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SECTION MEETINGS

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University of Minnesota	March 2, 1938
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9787 P

Experimental Infection of *Ornithodoros turicata* (Duges) with a Brazilian strain of *Trypanosoma cruzi* Chagas.

CHARLES M. WHEELER. (Introduced by C. D. Leake.)

From the George Williams Hooper Foundation for Medical Research in the University of California, San Francisco, California.

The transmission of *Trypanosoma cruzi* Chagas, the causative organism of Chagas Disease, is usually accomplished through the agency of the reduviid bug, *Panstrongylus megistus* Burm. *(Triatoma megista).* Craig and Faust[1] list several other arthropods as vectors of the trypanosome under experimental conditions including several species of Ixodoidea namely, *Amblyomma cajennense* (Fabr.), *Rhipicephalus sanguineus* Latr., and *Ornithodoros moubata* Murray. In addition Brumpt has shown the argasine tick, *Ornithodoros savignyi* (Audouin) capable of transmitting the organism in the laboratory.

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[1] Craig, C. F., and Faust, E. C., *Clinical Parasitology*, Philadelphia, 1937.

The following deals with the experimental infection of the tick, *Ornithodoros turicata* (Duges) with *Trypanosoma cruzi* Chagas and subsequent transmission of this species of trypanosome to normal white mice.

The outline of the experiment is as follows:

Data Concerning Ticks Used. Thirty clean larval ticks, *Ornithodoros turicata* (Duges) reared in the laboratory from a clean adult female tick taken in Texas, 1937.

Procedure. One white mouse, previously infected by means of an intraperitoneal inoculation of one cubic centimeter sterile saline to which was added a few droplets of fecal material taken from infective cone-nose bugs of the species *Triatoma protracta* Uhl.,* was placed in a special wire feeding cage and the ticks applied to the smooth skin of the abdomen of the animal.

Time of Feeding. First tick attached, 8:36 a. m.; detached, 9:25 a. m. Last tick attached, 9:38 a. m.; detached, 11:55 a. m.

Date of Feeding. August 20, 1937.

Date Ticks Molted. September 1, 1937.

Due to adverse temperature and humidity conditions only 5 of the 30 larval ticks molted successfully to the nymphal stage, the others died during the process of ecdysis. Upon examination of the larvae from time to time, after having engorged on the blood of the infected mouse, the larvae were observed to molt and further examination revealed that the ticks were still in the larval stage but considerably larger in size. In a survey of the literature dealing with this species of tick, the author has been unable to find a record stating definitely the number of larval instars during the life cycle of *Ornithodoros turicata* (Duges). In this instance the larval forms passed through 2 distinct larval instars and then molted again at which time the first nymphal stage appeared.

Infection of Normal White Mice. On September 8, 1937, the 5 nymphal ticks (referred to above) were placed on the abdomen of a normal white mouse but refused to feed. Three of these were subsequently macerated in a hard glass test tube and mixed with one cubic centimeter sterile saline solution. Two normal white mice were then inoculated intraperitoneally with the tick-saline solution—each mouse receiving one-half a cubic centimeter. Blood smears were prepared daily from the tail of these animals and examined under darkfield illumination and in smears fixed in methyl alcohol and stained with Giemsa stain. The 2 remaining ticks were saved for further study.

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* These insects acquired their infection by engorging on infective mouse blood containing a Brazilian strain of the organism, *Trypanosoma cruzi* Chagas.

Results. On September 29, 1937, (21 days following inoculation) both mice were positive for *Trypanosoma cruzi* Chagas in fresh blood preparations and in stained blood smears. The mice died 5 and 6 days later respectively. Similar experiments were conducted simultaneously in which a large number of the argasine tick, *Ornithodoros hermsi* Wheeler were used. To date all feeding and inoculation tests have given negative results. Further experiments are in progress with *Ornithodoros turicata* (Duges) and *Ornithodoros hermsi* Wheeler.

9788 P

High Gonadotropic Hormone Concentration in Pregnant Ponies.

H. H. COLE.

From the College of Agriculture, University of California, Davis.

Data from several hundred assays of mare sera for gonadotropic hormone point to the existence of an inverse relationship between body size and hormone content, draft mares usually having a lower concentration than the lighter Thoroughbred mares. This observation led to a study of the concentration in the pony. The sera of grade Welsh ponies were tested according to the method of Cole, Guilbert and Goss.[1] In some instances complete tests were not made, however, if the concentration was lower than 100 R.U. per cc. The results of tests on 10 ponies are shown in Table I. It is clear that a concentration of 200 to 400 R.U. per cc. is not at all uncommon, while we have never found sera testing 200 R.U. per cc. from Thoroughbred or draft mares. The range found is from 12 to 100 R.U. per cc. with a mean of 75 R.U. per cc. in the latter breeds. The data of Catchpole and Lyons[2] indicate that mustangs fall in the upper portion of this range. Thus the concentration in certain ponies is four times greater than has been found in the breeds mentioned. In serum testing 400 R.U. per cc. the rat unit represents approximately 0.25 mg. of dry matter. Thus it would seem that the blood of such ponies would be an excellent starting material for chemical studies.

The data of Table I show further that a variation in concentration occurs from year to year in the same mare. This is particularly

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[1] Cole, H. H., Guilbert, H. R., and Goss, H., *Am. J. Physiol.*, 1932, **102**, 227.

[2] Catchpole, H. R., and Lyons, W. R., *Am. J. Anat.*, 1934, **55**, 167.

TABLE I.

Gonadotropic Hormone in the Blood Serum of Pregnant Ponies.

Designation of mare	Days pregnant	Rat units per cc.	Designation of mare	Days pregnant	Rat units per cc.
1	61*	100	5	59	25
	73*	200		73	<100
	65	100			
	71	100	6	60	100
				71	<100
2	72*	50			
	84*	<100	7	59	100
	55	100		66	100
	62	100		73	<100
	71	200			
			8	62	100
3	61	200		82	<100
	66	400			
	75	266	9	58	200
				64	200
4	64	200 to 400			
	70	400	10	61	200
				69	200

*These tests were made in 1936; the remainder in 1937.

well demonstrated in pony 2 in which the concentration in 1937 was 4 times that of 1936 at comparable stages of pregnancy. We have similar observations for mares of other breeds but have not been able to relate this variation to any difference in physiological or nutritional state.

The data, though incomplete, indicate that the time of maximum concentration of the hormone in the pony falls between the 60th and 75th days following breeding. Apparently the concentration begins to drop somewhat earlier than is the case in thoroughbred and draft mares, in which the greatest concentration is usually found between the 70th and 80th days. In 3 instances, mares 6, 7, and 8, the concentration was lower after the 70th day than in preceding tests.

9789 P

Failure of Nicotinic and Yeast Nucleic Acids in "Filtrate Factor" Deficiency in Rats.*

GLADYS A. EMERSON AND HERBERT M. EVANS.

From the Institute of Experimental Biology, University of California, Berkeley.

An aqueous extract of fresh hog's liver will stimulate growth in rats, cure a specific dermatitis in chicks, cure black-tongue in dogs, and cure human pellagra, when fed in conjunction with diets that would otherwise produce a deficiency disease.

Elvehjem, Madden, Strong and Woolley[1] have shown that nicotinic acid (or its amide) will cure black-tongue. This finding has been confirmed.[2, 3] Likewise nicotinic acid has been shown with high probability to be the anti-human pellagric factor.[4] Results with chicks and rats have been negative.[5, 6]

Frost and Elvehjem,[7] however, obtained a definite growth response in rats by the addition of adenine nucleotide and nicotinic acid or its amide to their basal ration, K_{12}, supplemented with flavin.

The experiment herein reported was designed to test the possible growth-stimulating action of nicotinic acid and yeast nucleic acid when added to a diet deficient only in the factors supplied by liver filtrate. Diet No. 805 was employed:

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We wish to acknowledge the assistance rendered by the Federal Works Progress Administration (Project No. 7787).

The following materials were generously supplied:

Cod liver oil by E. R. Squibb and Sons.
Thiamin Chloride (Betaxin) and Riboflavin by Winthrop Chemical Company.
Hog liver by the H. Moffat Company.
Molasses by Waialua Agricultural Company.

[1] Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W., *J. Am. Chem. Soc.*, 1937, **59**, 1767.

[2] Dann, W. J., *Science*, 1937, **86**, 616.

[3] Street, H. R., and Cowgill, G. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 547.

[4] Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 405.

[5] Cook, C. A., Clarke, M. F., and Light, A. E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 514.

[6] Macrae, T. F., and Edgar, C. E., *Biochem. J.*, 1937, **31**, 2225.

[7] Frost, D. V., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **121**, 255.

Casein, extracted[8]	24
Sucrose	67
Fresh lard	3
Cod liver oil	2
Salts (McCollum 185)	4

Twenty-four female rats were placed at weaning on the above diet supplemented with 10 micrograms of thiamin chloride 6 times weekly.

After 4 weeks on the ration, all animals had plateaued in weight. They were then segregated, one littermate in each of 4 groups. All rats then received the following supplements 6 times weekly:

Thiamin chloride	10 micrograms
Riboflavin	40 micrograms
Molasses eluate	44 mg. total solids, equivalent to 4 gm. molasses

The eluate was assayed previously for vitamin B_6 potency.

The additional supplements were as follows:

Group I a—negative controls—no supplements.
I b—after 15 days, 3 of the above group received liver filtrate equivalent to 5 gm. original liver.
Group II —5 mg. nicotinic acid (Eastman) recrystallized to m.p. 228-230°.
Group III—5 mg. yeast nucleic acid (Eastman).
Group IV —5 mg. yeast nucleic acid + 5 mg. nicotinic acid.

After 15 days on the supplements all 4 groups had evidenced like growth responses, and 3 of the negative controls were given a previously assayed multiply adsorbed liver filtrate prepared according to Lepkovsky, Jukes and Krause,[9] except that 6 rather than 5, adsorptions were made. The total gains were as shown in Table I.

TABLE I.

Preparation fed 6 times weekly	No. of rats	Days after initial depletion period	Gain in gm.	Aver. daily gain, gm.
None	3	1-56	27	0.48
Liver filtrate equivalent to 5 gm. original liver	3	15-56	82	2.00
5 mg. nicotinic acid	6	1-56	26	0.46
5 mg. yeast nucleic acid	6	1-56	25	0.45
5 mg. yeast nucleic acid + 5 mg. nicotinic acid	6	1-56	20	0.36

Nicotinic acid and yeast nucleic acid, fed separately or combined, failed to stimulate growth in rats supplied with basal diet 805, thiamin chloride, riboflavin and vitamin B_6 as a molasses eluate.

A liver filtrate multiply adsorbed on fuller's earth to remove B_1

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[8] Halliday, N., and Evans, H. M., *J. Nutrition*, 1937, **13**, 657.

[9] Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

(thiamin chloride), riboflavin and vitamin B_6 produced an immediate growth response. The rôle of nicotinic acid as a component of the original filtrate may be masked by a double deficiency. It is possible that although adenine nucleotide is utilized, this is not the case with yeast nucleic acid, and it is also possible that the K_{12} ration fed by Frost and Elvehjem conceivably supplies factors lacking in our ration.

All rats except those receiving liver filtrate showed a non-specific type of skin lesions dissimilar to the florid acrodynia type of dermatitis characteristic of vitamin B_6 deficiency. The fur was dull and without luster.

Summary. Female rats received a diet in which all the known factors of the vitamin B_2 complex were supplied except that found in a multiply adsorbed liver filtrate. They failed to show a growth response on the addition of nicotinic acid, yeast nucleic acid, or a combination of the two to their ration. Liver filtrate, on the other hand, produced an immediate and marked stimulus to growth.

9790 P

Growth-Stimulating Action of Alpha Tocopherol.*

HERBERT M. EVANS, GLADYS A. EMERSON AND OLIVER H. EMERSON.

From the Institute of Experimental Biology, University of California, Berkeley.

It would now appear to be well established that in the rat a continuance beyond the fourth month of normal general body growth requires a nutritive factor which is either vitamin E or a substance closely related to it.[1-5] The growth-stimulating factor was found to be present in wheat germ and cottonseed oils and in their nonsaponifiable fractions, a distribution which parallels that of vitamin

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* Aided by grants from the Research Board and the College of Agriculture of the University of California and the Rockefeller Foundation of New York City.

We wish to acknowledge the assistance rendered by the Federal Works Progress Administration (Project No. 5778).

We wish to thank Merck & Company, of Rahway, New Jersey, for a stipend to one of us (G. A. E.).

[1] Evans, H. M., *J. Nutr.*, 1928-29, **1**, 23.

[2] Blumberg, H., *J. Biol. Chem.*, 1935, **108**, 227.

[3] Olcott, H. S., and Mattill, H. A., *J. Biol. Chem.*, 1936, **114**, lxxvii.

[4] Olcott, H. S., and Mattill, H. A., *J. Nutr.*, 1937, **14**, 305.

[5] Emerson, G. A., and Evans, H. M., *J. Nutr.*, 1937, **14**, 169.

E. Martin[6] believed that he had satisfactorily separated the growth-promoting and fertility-conferring effects, but took care to point out that the apparent difference might be a quantitative one, rather than due to two separate factors.

The results herein reported deal with the growth-stimulating action of alpha tocopherol. This compound was prepared as described by Evans, Emerson and Emerson[7] by hydrolysis of alpha tocopheryl allophanate (m.p. 158-160°). The purity of the compound seems established.

Female rats were reared from weaning on vitamin E-low diet 427[8] and had plateaued in weight at about 120 days of age. When 195 days of age, 6 animals were given, for 50 days, 6 times weekly, 1 mg. of alpha tocopherol dissolved in 80 mg. of ethyl laurate. Three controls received 80 mg. of ethyl laurate alone. The results are shown in Table I.

TABLE I.
Growth Response to Alpha Tocopherol of Rats Maintained on a Vitamin E-Low Ration.

Supplement 6 times weekly	No. of rats	Days	Average gain in wt., gm.
1 mg. alpha tocopherol in 80 mg. ethyl laurate	6	195-245	22
80 mg. ethyl laurate alone	3	195-245	0

The particular sample of alpha tocopherol when assayed with young female rats of proved sterility gave results as shown in Table II.

TABLE II.
Vitamin E Activity of Alpha Tocopherol.

Level fed single dose, mg.	No. of rats fed	No. of litters	% littering	Av. No. living young per litter	Av. wt. per rat, gm.	Dead young
1	5	2	40	9.5	5.4	0
3	4	4	100	5.5	5.3	6

Summary. Alpha tocopherol potent as vitamin E in a single dose of 1-3 mg. stimulated growth in rats that had plateaued in weight on a vitamin E-low diet.

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[6] Martin, G., *J. Nutr.*, 1937, **13**, 679.

[7] Evans, H. M., Emerson, O. H., and Emerson, G. A., *J. Biol. Chem.*, 1936, **113**, 319.

9791 P

Experiments on the Supposed Relation of Epinephrine Secretion to Hypertension.

J. M. ROGOFF,* E. MARCUS AND P. WASSERMAN.

From the Physiological Laboratory, University of Chicago.

Cannon and de la Paz[1] suggested that, in emotional excitement, "some of the adrenal secretion set free by nervous stimulation returns to the glands in the blood stream, and, within limits, stimulates them to further activity." If this occurs, it is conceivable that it might play a significant rôle in the production of a persistent elevation of blood pressure. However, such an "autogenous continuance of adrenal secretion," assumed to be important as an emergency function, would at the same time constitute a serious potential menace.

We made quantitative determinations of the rate of epinephrine secretion from the adrenals in dogs after intravenous administration of epinephrine. Ten to 500 times the average amount of epinephrine ordinarily secreted per minute (Stewart and Rogoff[2]) was injected. Epinephrine concentrations were determined with segments of rabbits' intestine. Some of the results were confirmed by the method described by Rogoff.[3] In some experiments the epinephrine was administered at a constant rate, in others it was given in a single injection to simulate an "outburst" from the adrenals as is alleged to occur during emotional excitement. Injection of 0.002 mg. per kg. body weight per minute caused an elevation of blood pressure in anesthetized dogs; striking effects were produced by the larger doses.

Constant injection of epinephrine in doses equal to 10, 20, and 50 times the average normal rate of liberation from the adrenals, did not influence the rate of epinephrine secretion from the glands; nor did such quantities measurably alter the epinephrine concentration in the adrenal vein blood. Similar results were obtained with doses up to 500 times the normal output although in some cases the epinephrine concentration in the adrenal vein blood was increased by the circulating epinephrine. Such increased concentration in the

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* Aided by the G. N. Stewart Memorial Fund and a grant from the Rosenwald Family Association.

[1] Cannon, W. B., and de la Paz, D., *Am. J. Physiol.*, 1911, **28**, 64.

[2] Stewart, G. N., and Rogoff, J. M., *Am. J. Physiol.*, 1923, **66**, 235.

[3] Rogoff, J. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 441.

adrenal blood closely corresponds with the epinephrine concentration in the systemic circulation and does not necessarily indicate increased secretion. If an increase does occur it is small and transient; when pressor response of a massive dose subsides, a transient diminution in the adrenal output is observed.

These tremendous doses of epinephrine are much greater than the amounts liberated from the adrenals under powerful stimulation. Stewart and Rogoff[4] observed increases up to 20 or 30 times the initial epinephrine output under the influence of certain drugs or electrical excitation of the splanchnic. This would correspond with the smaller amounts employed in the present experiments. We can confirm the observations of Dragstedt, *et al.*,[5] that constant injection of epinephrine in quantities corresponding to 10 times the normal average rate of secretion from the adrenals, or more, cannot be continued for long without serious consequences.

Following a single injection of a quantity equal to 25 to 50 times the amount ordinarily secreted per minute by the adrenals, epinephrine can no longer be detected in the systemic blood about 2 minutes after the injection. Approximately half of the injected epinephrine may be detected in the arterial blood if it is obtained within 30 seconds after injection. These observations on dogs agree with unpublished results on cats obtained in 1931 by Rogoff and Wasserman at Western Reserve University.

The much larger doses of epinephrine yielded similar results, although presence of detectable amounts of epinephrine in the blood sometimes persisted a little longer when large amounts were injected constantly for long periods. We found a loss of epinephrine from the blood in passing from the arterial to the venous course of the circulation. A similar loss was observed in the pulmonary circulation. In quantities that are within the limits of possible experimental increase of secretion by the adrenals, injected epinephrine very rapidly disappears from the circulation.

A few experiments were done in some of which acute hypertension was produced by renal ischemia following firm application of Goldblatt clamps, in others by ligation of the renal arteries. In none of the animals was the rate of epinephrine secretion from the adrenals different from that ordinarily found in normal dogs. Goldblatt[6] was able to produce chronic hypertension by renal ischemia after excision of one adrenal, denervation of the other and mechanical destruction of its medulla. This type of hypertension, therefore, apparently is independent of epinephrine secretion from the adrenals. Rogoff and Wasserman tested bloods from a small group of patients with hypertension (obtained through the courtesy of Dr. R. W. Scott at Cleveland City Hospital) and were unable to detect epinephrine in the specimens. Stewart[7] had previously reported a similar negative result.

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[4] Stewart, G. N., and Rogoff, J. M., *J. Pharm. Exp. Therap.*, 1919, **13**, 95, 183; *Am. J. Physiol.*, 1924, **69**, 605.

[5] Dragstedt, L. R., Prohaska, J. V., and Harms, H. P., *Am. J. Physiol.* (proc.), 1937, **119**, 298.

[6] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

These experiments lead to the conclusion that increased secretion of epinephrine from the adrenals, as a probable factor in causing persistent hypertension, lacks support of adequate quantitative experimental evidence. Our evidence is against the physiological concept which is a basis for the clinical procedure of surgical interference with the adrenal glands as a therapeutic measure for the relief of hypertension.

9792

Effects of Thyroxin and Female Hormone on One Phase of Saddle Feather Development.

RICHARD M. FRAPS. (Introduced by F. R. Lillie.)

From Whitman Laboratory of Experimental Zoology, University of Chicago.[*]

The c-isochrone has been defined by Fraps and Juhn[1] as the locus of points in the regenerated feather representing a given level of cell division around the annular collar of the feather germ. This locus appears as a constant configuration in each vane-half of the grown feather and is symmetrical with respect to a common transverse shaft level. If the barbs of either vane-half are in parallel array (at any uniform angle with the shaft), the c-isochrone is a straight line from shaft to margin, equidistant on shaft and a given barb from the point of union of that barb with the shaft. In Fig. 1, for example, barbs are laid out at right angles to the shaft, AB (apex of the feather at A), and the c-isochrone must form in this particular construction an angle of 45° with the shaft. Since the apex of the feather forms first, an apical-most c-isochrone, *Am* of Fig. 1, drawn from apices of the two barbs uniting at their bases to form the shaft (when these are in alignment with the shaft) identifies elements represented in the collar at the onset of feather growth. Successive c-isochrones taken at regular intervals in apico-basal order determine similarly the number of barbs simultaneously represented in the collar of the germ and are the source of other data treated by Juhn and Fraps[2] in the formation of a number of developmental relations. The subcutaneous injection of thyroxin or female hormone into the Brown Leghorn male or capon causes, among other things, marked changes in the contours of certain feathers, notably those of the saddle tracts. What appeared to be a limiting effect of thyroxin on contour was taken by Fraps and Juhn as evidence for the constant configuration of the c-isochrone itself, a deduction resting entirely upon the existence of an open ventral field† in saddle feather germs. It was suggested that this ventral field does not differentiate into definitive barb primordia in the normal saddle feather germ, but that it does so following injection of thyroxin in sufficient concentrations. The present paper records the results of further c-isochrone analyses, including previously unreported modifications effected by female hormone. The germinal relations corresponding to the c-isochrone measures reported here are described elsewhere.[3]

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[7] Stewart, G. N., *J. Exp. Med.*, 1911, **16**, 502.

[*] This investigation was supported in part by funds from a grant by the Rockefeller Foundation to The University of Chicago in aid of biological research.

[1] Fraps, Richard M., and Juhn, Mary, *Phys. Zool.*, 1936, **9**, 319.

The limiting effect of thyroxin on contour (and associated variables) is a convenient basis of comparison of other modifications. Let Fig. 1 represent a section from one vane-half of a normal saddle feather upon which certain hormone modified contours are superimposed. The part of the figure shown in heavy lines to give the contour *Amnop* represents approximately the main vane region of the unmodified saddle feather (by main vane region is meant that part of the feather from apex to levels at which down or "fluff" is produced). Changes in spacing of barbs on the shaft do not alter the relative magnitudes of interest here and are not shown in the figure.

Following a single injection of 10 mg. thyroxin in aqueous solution, the saddle feather of the male or capon takes on approximately the contour outlined by *ost* of Fig. 1. The c-isochrone drawn from *d* and intersecting the normal contour at *o* indicates barb levels

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[2] Juhn, Mary, and Fraps, Richard M., *Phys. Zool.*, 1936, **9**, 293.

† The *ventral field* here denotes the ventral collar region of the regenerating feather germ at the opposed faces of which barb apices arise (see Fraps[3]). The ventral triangle (Lillie and Juhn[4]) is considered a special case in the present discussion.

[3] Fraps, Richard M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 206.

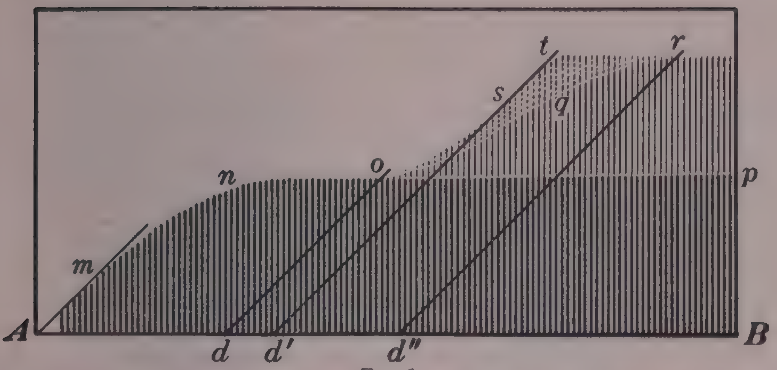

FIG. 1.

Section of one vane-half of a saddle feather from male or capon Brown Leghorn with contours induced by thyroxin and female hormone superimposed. The diagram is simplified by omission of certain striking effects which do not enter directly into relations considered here (see Juhn and Fraps,[5] Fraps and Juhn[1]). Barbs are at right angles to the shaft, *AB*, with feather apex at *A*. Contour of normal saddle feather, *Amnop;* following thyroxin injection, *ostr;* following female hormone injection, *oqr*. The diagonals *Am*, *do*, *d′t*, and *d″r* are c-isochrones defining in the order given the apical barb formation, the level of initial hormone effect (for both curves), the level of simultaneous reaction to thyroxin, and the level of maximum female hormone effect.

simultaneously represented in the collar at the time of initial thyroxin effect. All barb apices and barb segments basal to this locus originated or were grown following thyroxin effect. Considering only the origin of barb apices, the effect of thyroxin falls into 3 distinct phases. From *o* to *s*, Fig. 1, the number of apices formed per mm. axial growth of the germ increases rapidly; the measure of this effect is most simply seen as the number of barb apices lying between successive c-isochrones, supposing these to be drawn into the figure at small uniform intervals basal to c-isochrone *do*. The second phase of effect is indicated in Fig. 1 by the alignment of barb apices between *s* and *t* along the locus of the single c-isochrone, *d′t*. The third phase is marked by an abrupt break in the number of apices formed per mm. axial growth of the germ and appears basal to *t* in the figure.

Assuming the accuracy of the c-isochrone relationship, the first phase of thyroxin effect means that increasing numbers of barb apices are formed in the ventral field with increasing thyroxin concentration. The second phase signifies the simultaneous formation of a greater or lesser number of primordia at a critical level in thyroxin concentration. This phase is apparently analogous to formation of barb apices at the feather apex (*A* to *m*, Fig. 1); Lillie and Juhn[4] have presented evidence indicating that these apical-most barb primordia are of simultaneous origin in the germ. The third phase (basal to *t*) must occur if the ventral field has been suddenly and completely occupied with barb primordia, since barb primordia can now arise only from the faces of the usual ventral triangle as this is restored following formation of a primordium by growth from the base of the feather germ. This interpretation of events is supported by examination of germs in process of regeneration (Fraps[3]).

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[4] Lillie, Frank R., and Juhn, Mary, *Phys. Zool.*, 1932, 5, 124.

The increasing length of barbs the apices of which have formed in the ventral field is clear if we suppose that successive barb apices arise serially in strict dorso-ventral order around each half of the collar. This supposition is in accord with observations of Lillie and Juhn[4] on completion of collar complements following formation of the limited apical primordia of simultaneous origin. Many saddle feathers show striking contour modifications in the region basal to *n* of Fig. 1 following thyroxin injection. These changes are due to changes in frequency with which barbs join the shaft (Juhn and Fraps,[5] Hardesty,[6] Fraps and Juhn[1]), and have no effect upon differentiation of apices in the ventral field.

Injection of thyroxin in 1 to 5 mg. doses (single injections) produce modifications ranging from slight convexities in contour to fairly extensive shifts toward (but never attaining) the contour *ost* of Fig. 1. In general, then, thyroxin appears to cause differentiation of the ventral field in proportion to amounts injected over rather wide limits, with simultaneous differentiation of the entire field into definitive barb primordia as a limiting condition of effect.

Curve *oqr* of Fig. 1 represents the approximate contour of a saddle feather following modification by injection of female hormone (1st to 3rd days, 400 r.u. daily; 4th to 6th days, 800 r.u. daily; 7th to 9th days, 1200 r.u. daily). The c-isochrone *do* indicates the level of initial hormone effect, c-isochrone $d''r$ the level of maximum hormone effect. The total number of barbs cut by $d't$ is of the same order as the number cut by $d''r$, indicating that maximum effects of thyroxin and female hormone are approximately equivalent on the ventral fields in these 2 instances. Thyroxin, however, produces its maximum effect (following 10 mg. injections) much more rapidly, as is seen by comparing growth increments dd' and dd'', Fig. 1. Whether sufficiently high concentrations of female hormone can reproduce the rapidity of effect obtained following thyroxin injection (10 mg.) is not known at present.

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[5] Juhn, Mary, and Fraps, Richard M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1185.

[6] Hardesty, Mary, *J. Exp. Zool.*, 1935, **71**, 389.

The effects obtained with female hormone in saddle feathers of the Brown Leghorn male or capon are of particular significance in view of the fact that the same hormone in equal or greater concentrations produces in breast feathers negligible or very slight effects that can be referred to the ventral fields (or ventral triangles) of these feathers. This difference in reactivity of feathers from the 2 tracts must involve, in some part at least, differences in the ventral fields of the germs if the interpretation of effects followed here is sound. Observational evidence for the existence of the postulated differences is given elsewhere (Fraps[3]).

Hardesty[6] has reported the presence of additional barb primordia in breast feather germs of the guinea fowl within 24 hours after injection of thyroxin (4 mg.). Thyroxin may produce a similar (though probably not extensive) effect in certain regions of breast feathers of the Brown Leghorn; however, the lack of appreciable reaction to female hormone in Brown Leghorn breast feathers points rather to characteristic species differences in the 2 birds, as do many other observations recorded by Hardesty.

It is more than likely that changes in growth vectors, in the size of barb primordia, in the slopes of barb primordia at the ventral limits of the collar, and perhaps in other factors contribute in part toward effecting results described in this paper. These changes, however, could not account for the approximation to simultaneity in origin of a certain number of barb apices; the simultaneous formation of primordia thus remains the critical datum indicating the existence of an open ventral field in normal saddle feather germs of the Brown Leghorn male or capon. Assuming the validity of this critical relationship, the increasing order of effect with increasing hormone concentrations (both thyroxin and female hormone) indicates further that the ventral field is subject to threshold differentials increasing in dorso-ventral order. Lillie and Juhn[7] have compared the properties of the ventral field or triangle ("formative center") with the properties of the primitive streak or blastopore in the formation of the axis of the embryo. The application of c-isochrone procedures to the regenerated feather appears to make possible the accurate quantitative analysis of this "organizer" and its field with respect to its reaction under varying general physiological conditions.

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[7] Lillie, Frank R., and Juhn, Mary, *Science*, 1937, **86**, 38.

9793

Germinal Basis of Thyroxin and Female Hormone Effect on Barb Origin in Saddle Feathers.

RICHARD M. FRAPS. (Introduced by F. R. Lillie.)

*From Whitman Laboratory of Experimental Zoology, University of Chicago.**

The subcutaneous injection of thyroxin or female hormone into the Brown Leghorn male or capon brings about modifications in saddle feathers which, on the basis of c-isochrone relations, signify the origin and growth of definitive barb primordia in a previously undifferentiated ventral field of the germ (Fraps and Juhn,[1] Fraps[2]). With sufficient concentration of thyroxin, a greater or lesser number of barb apices appear to arise practically simultaneously. Female hormone brings about only slight changes of a similar nature in breast feathers, suggesting that the ventral regions of these germs normally carry a full complement of barb primordia. The only direct observation bearing on this indicated difference in germinal organization was made by Lillie and Juhn,[3] who noted that the unoccupied ventral region of saddle feather germs appeared to be considerably wider than the comparable region in breast germs; Lillie and Juhn, however, attributed this difference largely to the lowness of ridges in saddle germs. A comparison of breast and saddle feather germs prepared before and following injection of thyroxin has therefore been made with a view toward establishing the embryonic basis of the ventral differentiations indicated by c-isochrone analyses of regenerated feathers.

Figs. 1-5 represent, rather schematically, the ventral regions of regenerating germs split along their dorsal faces and spread into the plane of the paper. Apical levels are cut away (excepting in Fig. 1), as are also the undifferentiated germ bases. The sloping lines indicate barbs and barb primordia visible in the several preparations. Increasing diameters of barb bases with increasing distances of bases from sites of origin are not shown in the figures, nor are differences in slopes of barb primordia in different germs or regions. The essential relations illustrated by the several figures follow.

Fig. 1. Germ of breast feather, male or capon, showing open

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* This investigation was supported in part by funds from a grant by the Rockefeller Foundation to the University of Chicago in aid of biological research.

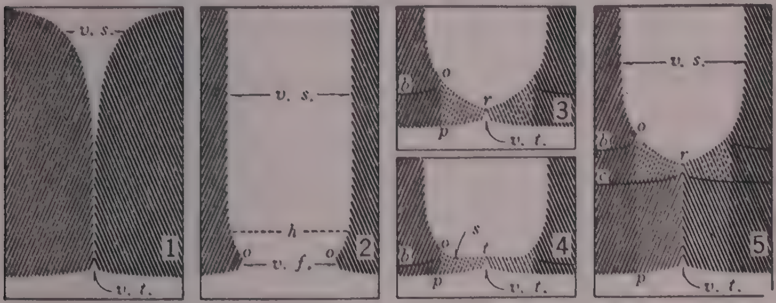
[1] Fraps, Richard M., and Juhn, Mary, *Phys. Zool.*, 1936, **9**, 319.

[2] Fraps, Richard M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 201.

[3] Lillie, Frank R., and Juhn, Mary, *Phys. Zool.*, 1932, **5**, 124.

ventral area or strip, *v. s.*, extending a short distance below feather apex. This open area decreases rapidly in width with axial growth of the germ, and at an early stage barb primordia come to arise from opposite faces of a typical ventral triangle, *v. t.* Injection of female hormone modifies only slightly breast feathers in the stage of development here represented. There is some evidence that supranormal concentrations of thyroxin cause differentiation in the open apical levels of the breast germ (*v. s.* of Fig. 1 when this region lies at the base of the germ), but comparison of mounted feathers will be required to clear up this point.

The serial origin of barb primordia from a ventral triangle is characteristic of most feather germs from completion of apical levels to formation of the after-feather (Lillie and Juhn[3, 4]).



FIGS. 1-5.

Sections from ventral regions of regenerating feather germs, each prepared by splitting the germ along its dorsal axis and spreading into the plane of the paper.

FIG. 1. Breast feather germ, near apex of feather: *v.s.*, ventral strip (compare Fig. 2); *v.t.*, ventral triangle.

FIG. 2. Saddle feather germ, uninjected male or capon, at main vane levels: *v.s.*, ventral strip; *v.f.*, ventral field; *o*, sites of origin of definitive barb primordia; *h*, distal collar level.

FIG. 3. Germ of Fig. 2 following action of thyroxin and growth of base of Fig. 2 to level *b*, Fig. 3. The locus of definitive barb apices forming in the ventral field is indicated by *or*; *v.t.*, ventral triangle; *op*, projected face of ventral field of Fig. 2.

FIG. 4. Same as Fig. 3, but assuming more rapid thyroxin effect on ventral field: *os*, first phase of thyroxin action; *st*, barb apices theoretically forming simultaneously in left collar half.

FIG. 5. Germ represented in Fig. 3 following growth raising base line of Fig. 3 to level *c* of Fig. 5, and assuming maintenance of thyroxin concentration.

Fig. 2. Saddle feather germ from an uninjected male or capon. A longitudinal mid-ventral strip, *v. s.*, separates visible (definitive) barb apices of opposite vane-halves. Basal levels of this open area, approximately levels below *h*, lie in the collar of Lillie and Juhn (1932) and may be denoted the ventral field, *v. f.* Definitive barb

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[4] Lillie, Frank R., and Juhn, Mary, *Science*, 1937, 86, 38.

apices become visible (or differentiated) at opposite faces, *o*, of the ventral field. At proximal levels of the main vane structure the ventral field becomes narrower and is finally closed; barb primordia then arise from opposite faces of a ventral triangle until formation of the after-feather (Lillie and Juhn[4]).

Fig. 3. Saddle feather germ comparable to that of Fig. 2 following reaction to thyroxin in sufficient concentration to close the ventral field. The locus of barb bases shown in Fig. 2 becomes the transverse locus, *b*, and indicates approximately the axial growth occurring during differentiation of the ventral field. Definitive barb apices arise successively from *o* to *r* (in each collar limb), with formation of the ventral triangle, *v. t.*, when the ventral field is completely occupied. The first stages of this process are demonstrated by comparison of the distance between faces of the ventral field (*o* to *o*, Fig. 2) in normal and thyroxin treated germs.

Fig. 4. (Theoretical case.) The same relations described for Fig. 3, but assuming more rapid action of thyroxin in differentiation of the ventral field. Apices between *o* and *s* arise successively and with increasing rapidity as thyroxin concentration increases; apices between *s* and *t* are presumably of simultaneous origin. It has not been possible to identify positively this limiting effect in the germ. Possibly barb primordia of simultaneous origin form vertically in the ventral field, as do apical-most complements (*cf.* Lillie and Juhn[3]), but such a formation has not been observed. The lower level of the base line, *b*, compared with the same locus of Fig. 3 indicates the lesser growth required (in Fig. 4) during complete differentiation of the ventral field in this case.

Fig. 5. A germ such as represented in Fig. 3 after growth has raised the locus of barb bases (of Fig. 3) to the level *c*. The concentration of thyroxin is assumed to remain at a sufficiently high level to maintain the ventral triangle shown in Fig. 3. The approximate increase in length of barbs laid down following thyroxin over barb lengths of the normal male or capon feather is represented by segments lying between *op* and *or*.

In saddle feather germs from Brown Leghorn, Black Minorca, or Barred Rock males it has proven impossible to identify in the split preparation barb primordia or apices between such a contour as is represented in Fig. 2. In cross-sections through the collars of comparable germs, however, the entire circumference is seen to be occupied by barb primordia or very similar structures.† This observa-

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† The author wishes to express his appreciation of Professor Frank Lillie's generosity in placing at his disposal the series of cross-sections of saddle germs on which the observations reported here are based.

tion may be taken to mean that the ventral field of the split preparation (Fig. 2) carries normally differentiated primordia which in these preparations cannot be made visible, but a number of considerations make this conclusion highly improbable. Firstly, the apex of a barb primordium rises practically vertically above its site of origin in the collar; if *definitive* barb apices were originating from a ventral triangle in the saddle germ they would become visible, after differentiation, in approximately the center of the ventral strip instead of at its faces. Secondly, cross-sections show a considerably greater number of barb primordia (or apparent primordia) than are indicated by c-isochrone counts in regenerated feathers at comparable levels. Thirdly, the number of barb primordia (or apparent primordia) visible in cross sections of normal saddle germs is of the same order as is the number of barbs lying on c-isochrones through levels of maximum thyroxin or female hormone effect,[2] indicating perhaps (but not conclusively) that the hormones bring about definitive differentiation of rudimentary structures. Fourthly, the apparent primordia of the ventral collar levels become highly attenuated above levels of growth in the germ. Finally, the order of events characterizing the origin and development of definitive barb primordia in the ventral field following thyroxin injection (Figs. 3 and 5) makes it practically certain that definitive barb apices and corresponding segments do not occupy this region in the normal saddle feather germ.

The width of the ventral field of Fig. 2 is somewhat narrower than the distal ventral strip. This seems to be a common condition, even at those levels of the germ producing practically uniform regions of the feather vane. There is a suggestion in this out-curving of barb apices that secondary processes of dedifferentiation may involve not alone rudimentary structures invisible in split preparations, but also limited lengths of primordia identifiable in these preparations. As against this, it must be recognized that a part or all of the structures appearing in cross-sections of the ventral field may conceivably be independent of the visible differentiations of split preparations. In either event, however, the ventral field seems beyond reasonable doubt to constitute the embryonic basis for the rapid or simultaneous formation of definitive barb apices indicated by c-isochrone analysis of feathers grown under supra-normal concentrations of thyroxin.

Hypothyroidism in the Brown Leghorn male (as well as in the male of other breeds) is associated with increased length of feather in proportion to width, and restriction of barbulation in saddle and other feathers (Greenwood and Blyth,[5] Parkes and Selye[6]). These changes, taken in connection with the effects of hyperthyroidism reported here, suggest a relative increase in extent of the ventral field of saddle feathers grown under conditions of hypothyroidism. The appearance in the breast tracts of longer, saddle-like feathers with restricted barbulation following thyroidectomy is in all probability likewise associated (among other germinal changes) with a ventral field of greater or lesser extent. It should be a matter of considerable interest to alter experimentally the balance of other hormones in thyroidectomized birds in order to test the specificity of thyroxin in determining and maintaining the limited ventral triangle of feather germs of different tracts.

9794 P

Evaluation of Germicides by a Manometric Method.[*]

J. BRONFENBRENNER, A. D. HERSHEY AND J. A. DOUBLY.

From the Department of Bacteriology and Immunology, Washington University School of Medicine, St. Louis, Mo.

The need for a method capable of evaluating germicides for chemotherapeutic use is universally recognized. Obviously, the first requirement is that the method should simulate as nearly as possible the conditions met in the animal body. In addition it should be relatively simple and should yield information regarding the action of the test substance on bacteria, and on animal tissues, in terms which would permit a direct comparison of the respective effects. The method of Lambert,[1] employed more recently by Salle,[2] does not seem to fulfill these requirements, since the parasitotropic and organotropic properties of the disinfectant are tested under profoundly different conditions. Moreover, it is based on a tissue-culture technic which is time consuming and laborious.

The manometric method proposed here has proved simple, rapid, and remarkably flexible. It permits the conditions of testing to be modified at will, and at the same time allows wide latitude in the

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[5] Greenwood, A. W., and Blyth, J. S. S., *Proc. Roy. Soc. Edinb.*, 1929, **49**, 313.

[6] Parkes, A. S., and Selye, H., *J. Genetics*, 1937, **34**, 297.

[*] Aided by a grant from the Blue Line Chemical Company, St. Louis, Mo.

[1] Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 683.

[2] Salle, A. J., McOmie, W. A., and Schechmeister, I. L., *J. Bact.*, 1937, **34**, 267.

choice of animal and bacterial test-subjects. Our experience with it has been limited so far to comparison of the effect of certain disinfectants on the adult mouse's liver, and on suspensions of *Bacterium coli* to which comparable amounts of protein in the form of horse serum were added. The depressant effect on the rate of oxygen-consumption in glucose-succinate buffer was taken as a measure of destructive action in either case.

The curves in Fig. 1 show the type of data obtained. These were evaluated by replotting the percentile inhibition of rates of oxygen-uptake attained during the arbitrarily chosen interval between the 15th and 20th minute, against concentration of disinfectant, and reading off the interpolated value for the concentration giving 50%

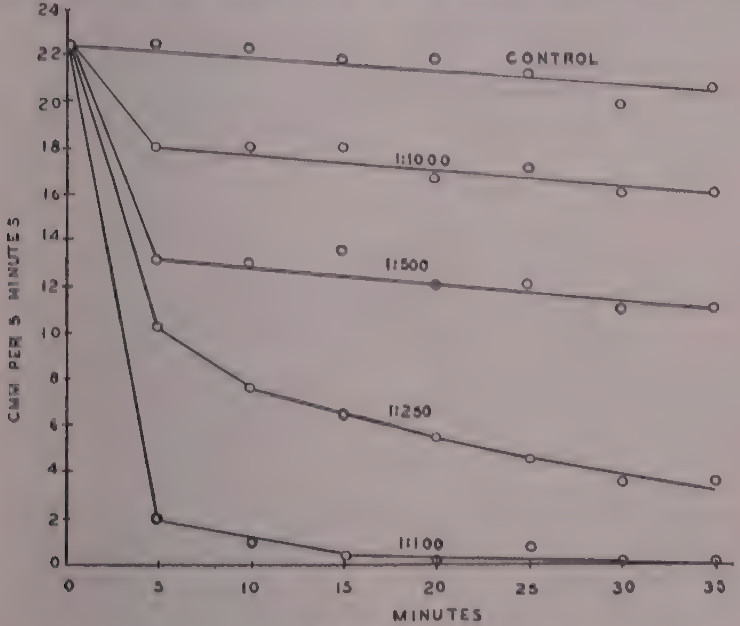

FIG. 1.

The Effect of Phenol on the Rate of O_2-consumption of Mouse Liver Cells.

The amounts of O_2 consumed in the test mixtures during successive 5-minute intervals, as measured in the Warburg Barcroft apparatus, are plotted against time following the addition of disinfectant.

For the test with liver cells, 0.1 gm. minced mouse liver in 2 ml. saline, 1 ml. M/10 phosphate buffer of pH 7.4, and 1 ml. 5% sodium succinate are placed on the floor of the vessel, with 1 ml. of aqueous solution of disinfectant in the side arm.

For the test with bacteria, approximately 3 × 10^9 organisms from centrifugalized 24-hour cultures of *Bact. coli* in broth, are placed in the vessel with 1.6 ml. saline, 0.4 ml. horse serum, 1 ml. M/10 phosphate buffer of pH 7.4, and 1 ml. 5% glucose, the disinfectant being added as before from the side arm.

In the control mixtures, water is substituted for the disinfectant.

inhibition (C). These concentrations could then be used to calculate the "phenol-coefficient" (C_{phenol}/C_x) for *Bact. coli*, and the "toxicity-index" ($C_{bact.}/C_{liver}$) for liver-cells. These values, for our series of disinfectants, are shown in Table I.

TABLE I.
Comparative Values Obtained by the Manometric Method for Representative Disinfectants.

Disinfectant	C_{bact} %	C_{liver} %	Phenol Coefficient $\frac{C_{phenol}}{C_x}$	Toxicity Index $\frac{C_{bact}}{C_{liver}}$
Phenol	.26	.23	1.0	1.1
Iodine (tincture)	.0425	.0165	6.1	2.6
Mercuric chloride	.00085	.004	306	0.21

Further work will correlate these data with those given by the usual methods, and by animal test, for several representative disinfectants.

9795

Comparison of Vitamin B_1 Content of Raw and Evaporated Milk by the 10-Day Bio-Assay Method.

AMY L. DANIELS. (With the coöperation of Mary F. Deardorff and Dorothy R. Linn.)

From the Iowa Child Welfare Research Station, State University of Iowa.

The amount of vitamin B_1 destroyed in the process of dehydration and sterilization involved in the production of evaported milk has been variously reported. Hartwell,[1] by means of lactation tests, found approximately a 50% destruction in some of the milks tested, 33 cc. of fresh cow's milk being almost enough and 50 cc. quite sufficient to meet the needs of the lactating rat and 6 young, when used in conjunction with the basal ration; whereas 75 cc. of evaporated milk was found to be too little. Daniels and Brooks,[2] and Daniels, Giddings, and Jordan,[3] using a similar technique with a slightly modified diet, obtained similar results. Feeding tests with pigeons (Donath,[4] and Daniels and Brooks[2]) also indicated that the evaporated milks tested contained approximately 50% less vitamin B_1 than the raw milk used in comparative tests.

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[1] Hartwell, G. A., *Biochem. J.*, 1925, **19**, 226.

[2] Daniels, A. L., and Brooks, L., PROC. SOC. EXP. BIOL. AND MED., 1927, **25**, 161.

[3] Daniels, A. L., Giddings, M. L., and Jordan, D. P., *J. Nutr.*, 1929, **1**, 455.

Samuels and Koch,[5] on the other hand, by 2 different methods: a modification of the Hartwell[1] lactation test, and a direct feeding procedure, found only about one-sixth less vitamin B_1 in the evaporated milks, while Dutcher, Francis and Combs[6] believe the difference in the potency of raw and evaporated milk to be so slight as to be negligible. These authors state: "Vitamin B is not readily destroyed by the evaporation process used, and only under unusual conditions would we expect the deficiency of commercial evaporated milk to be due to the process of manufacture."

The lack of unanimity in these reports has led to a study of the vitamin B_1 content of raw Guernsey milk (4.5% fat) and commercial evaporated milk (diluted 1:1) by a modification of the 10-day bio-assay method of Schlutz and Knott.[7] Young rats at weaning were fed a purified vitamin B_1-free ration consisting of: 20 gm. triple precipitated alcohol-extracted casein, 54.5 gm. cane sugar, 19 gm. Crisco, and 3.5 gm. Wesson's salt mixture.[8] In addition, each animal received 2 drops of cod liver oil. The other essential components of the vitamin B complex were supplied in certain of the tests by giving each day 0.5 gm. of whey* autoclaved 2 hours at 120°C. On the assumption that raw milk at the levels fed, contained enough of these other vitamin B complexes, some tests were made with raw milk and the basic ration without the supplement in order to rule out the influence of possible small amounts of vitamin B_1 in the autoclaved whey. The tests with evaporated milk included the 0.5 gm. autoclaved whey. In other tests, in order to make sure that vitamin B_1 only was concerned in the growth stimulation, assay animals were given the basic ration with evaporated milk (diluted 1:1) in amounts comparable to those used in the raw milk studies, and in addition, sufficient crystalline B_1 (Betaxin†) to make a daily gain of between one and 2 gm.

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[4] Donath, W. F., *Mededeelingen van den Dienst der Volksgezondheid in Ned. Indie*, 1929, **2**, 152.

[5] Samuels, L. T., and Koch, F. C., *J. Nutr.*, 1932, **5**, 307.

[6] Dutcher, R. A., Francis, E., and Combs, W. B., *J. Dairy Sci.*, 1926, **9**, 379.

[7] Schlutz, F. W., and Knott, E. M., *J. Nutr.*, 1936, **12**, 583.

[8] Wesson, L. G., *Science*, 1932, **75**, 339.

* Supplied through the courtesy of the Western Condensing Company, San Francisco, California.

† Supplied through the courtesy of the Winthrop Chemical Company, Inc., New York.

The autoclaved whey supplement in the amounts fed contained no vitamin B_1 or so little that it was without influence on the test animals. The control animals receiving the basic diet with this supplement, died in from 28 to 32 days without manifesting any of the reported symptoms of polyneuritis.

Subsequent to our first tests, animals were again depleted by the technique described, and when holding, were tested during second and third periods; thus the averages of the initial weights of the animals are slightly higher than designated in the original method. This seemingly has not influenced the results. Provided an animal is in the rapidly growing stage and is adequately depleted, his initial weight is not of major concern. The raw milk tests were made in June and July, whereas the evaporated milk tested, which was put out by the same manufacturer, was obtained from different lots, one purchased in the open market in July, and a second and third in December and January.

According to the vitamin B_1 assay method used, a unit of vitamin B_1 is the amount which makes possible a gain of 1 gm. per day in an assay animal during a 10-day test period when the rate of gain has been limited to between 1 and 2 gm. a day. Seventeen test animals receiving the basic diet with no supplement gained during the assay period an average of 1.45 gm. per day when 7 cc. of raw milk were fed, whereas 13 rats receiving the basic diet with the addition of 0.5 gm. of autoclaved whey per rat per day gained 1.5 gm. per day with an average ingestion of 7 cc. of raw milk per day. The results by these methods are within the limits of experimental error and indicate that 5.3 cc. (average) of the raw milk tested contained 1 unit of vitamin B_1. Similar tests with evaporated milk show that over twice as much, namely, 12.6 cc. was needed to produce the same amount of gain in the test animal. These findings are further emphasized by the results with the evaporated milk and crystalline B_1. In order to obtain comparable growth when equivalent amounts of raw and evaporated milk, 7 cc. respectively, were fed, with the autoclaved whey supplement it was necessary to add 4.0γ of crystalline B_1 which assays 5.7γ per unit.[9]

An estimation of the amount of the vitamin B_1 destroyed in the evaporated milk tested on the basis of the raw milk assayed, indicates a loss of 60%. These findings are in accord with those of workers (Hartwell, Donath, and Daniels, *et al.*) who have not used yeast as such or autoclaved, as part of the basic test ration. Bio-assays of raw milk with the basic ration and a 0.5 gm. autoclaved

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[9] Unpublished data.

TABLE I.
Ten-day Bio-assay of Raw and Evaporated Milk.

No. Rats	Milk		Supplement		Weight		Wt. gain per day gm.	Milk for 1 gm. gain	
	Kind	Av. per day cc.	Kind	Amt. gm.	Initial gm.	End gm.		Av. cc.	Stand. Error
13	Raw	7.8	Whey†	0.5	84.0	98.9	1.50	5.2±1.6	.42
17	,,	7.9	—	—	79.3	93.8	1.45	5.4±0.9	.94
4	,,	6.8	Yeast‡	0.5	82.3	104.8	2.25	3.0 —	—
26	Evap.*	21.2	Whey†	0.5	80.5	97.3	1.68	12.6±2.0	.39
8	,,	7.2	Whey† Betaxin§	0.5 4.0γ	70.1	85.2	1.51	— —	—

*Evaporated milk diluted 1:1.
†Autoclaved 2 hr. at 120°C. natural pH.
‡Autoclaved 6 hr. at 120°C. natural pH.
§Betaxin assays 5.7γ per unit when basal B_1 free diet is supplemented with 0.5 gm. autoclaved whey per day. (Unpublished data).

yeast (6 hours at 120°C.) supplement, gave higher values for the raw milk than when no supplement or when autoclaved whey was used (3.0 cc. against 5.3 cc. for one unit of vitamin B_1), suggesting that the higher values obtained when autoclaved yeast is used to supply the other components of the vitamin B complex are due to the presence of some vitamin B_1 in the supplement.

It is concluded that commercial evaporated milk contains about 60% less vitamin B_1 than an equivalent amount of raw summer milk.

9796 P

Reduced Latent Time of Blocking of the Berger Rhythm to Light Stimuli.

JOHN R. KNOTT. (Introduced by L. E. Travis.)

From the Department of Psychology, State University of Iowa, Iowa City, Iowa.

Recent reports[1, 2] have indicated that when a subject is instructed to respond manually to a light stimulus the latent time of blocking of the Berger Rhythm (the *a*-wave of the electroencephalogram) is shorter than it is when no response is made. One of these reports[2] includes a correlation of .37 ± .09 between reaction time and latent time of blocking. This might be held as indicative of a relationship between "readiness" of the subject and the blocking time.

The present report is concerned with the same general phenomenon, but with a different emphasis in the approach. The aim here is to discover, if possible, the factors involved in the reduction of the latent time of blocking.

A series of experiments was run to verify the results of the previous investigators. Ten normal adult subjects were used. Standard equipment for recording included resistance-capacitance coupled amplifiers driving an ink-writing oscillograph. Plate electrodes were placed on the left occipital area and on the lobe of the left ear. The stimulus lights were of an intensity of 0.3 cp. Response, when made, was by a break key inserted in the light circuit and activating the signal magnet of the ink-writer.

The verification experiments involved 3 situations. (1) A situation of no response. (2) A situation demanding discrimination

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[1] Travis, L. E., Knott, J. R., and Griffith, P. E., *J. Gen. Psychol.*, 1937, **16**, 391.

[2] Jasper, H. H., and Cruikshank, R. M., *J. Gen. Psychol.*, 1937, **17**, 29.

(response to one and only one of 2 lights). (3) A situation demanding speed (response to either of 2 lights).

The results may be summarized in Table I.

TABLE I.

Condition	AM (sec.)	SD (sec.)	N (stimulations)
No Response	.27	.11	500
Response$_1$ (Emphasis on discrimination)	.24	.07	553
Response$_2$ (Emphasis on speed)	.21	.12	686

The differences between the first condition and the second and third conditions were statistically significant, as was the difference between the second and the third conditions. The absolute magnitude of reduction appears slight, but the relative magnitude of reduction is 11% and 22%, respectively, for conditions 2 and 3, as compared with condition one.

In an attempt to determine the "cause" of this reduction 4 control experiments were run. To rule out increased sensitivity of the eye through dark adaptation, experiments were run in a reverse order (*i.e.,* 3, 2, 1,). The results held as presented in the table above. To rule out, possibly, effects from other cortical fields impinging upon the visual field, a tone of 180 cycles, about 85 db above threshold was impressed upon the subject while light stimulation was given. No reduction was observed. A third attempt was made on the supposition that imagery might be operating to pre-sensitize the visual region, leading to reductions of the order found. A uniform visual field (diffusely illuminated opal glass hemisphere) of low intensity was presented, upon which a brighter field aperiodically was flashed. Although this part of the study is still in a preliminary stage, the latent time of blocking appears to be longer than when the background illumination is absent. The fourth attempt was made on the lines of accepted neurophysiological theories of mental set, that there is a muscle strain involved. Hence, 3 subjects supported 5 pounds of lead in the palm of each hand (elbows on table, hands 10″ above table top) during light stimulation. The results indicate an absolute reduction of 0.02 sec., (which is a relative reduction of 8%, *i. e.,* from 0.25 to 0.23 sec.). This difference approaches statistical significance, the critical ratio being 2.6. Further experiments on this line, with increase in the weight supported (and consequently more widely spread proprioceptive stimulation, approaching that in actual "set") are now in progress.

9797

Accessory Factors in Lactic Fermentation.

Otto Rahn and C. P. Hegarty.

From the Laboratory of Bacteriology, New York State College of Agriculture, Cornell University, Ithaca, N. Y.

A previous paper[1] had shown that the rate of lactic-acid formation by *Streptococcus lactis* can be measured by placing the cells, obtained from a 12-hour culture by means of a centrifuge, into a buffered glucose solution and determining the acid by titration. In all experiments mentioned in the previous paper, 0.5% peptone had been added to the buffered glucose solution; this was essential for a rapid rate of fermentation. The present paper is a study of the rôle of peptone or other accessory factors.

Washing of Cells. In order to study the effect of accessory compounds, it was necessary to remove any traces of peptone or other compounds of the original medium from the cells. This was accomplished by resuspending the centrifuged cells in buffer, using an egg beater, centrifuging again, and repeating this operation at least once. The cells from 250 cc. of culture were then suspended in 50 cc. of phosphate buffer plus 2% glucose, and the acid was titrated every half-hour. In one experiment, the cells were tested after each washing in buffered glucose solution without and with peptone. Since the number of cells after each washing was different, the rate of fermentation is measured by the fermenting capacity (abbreviated F.C.) *i. e.*, mg. acid per cell per hour, expressed in units of 10^{-10} mg. The result of washing is shown in Table I.

TABLE I.
Fermenting Capacity.

	Without peptone	With .5% peptone	Increase by Peptone %
Unwashed	1.72	6.14	256
Washed once	1.22	6.53	435
” 2 times	1.81	7.46	313
” 3 ”	1.81	8.00	342
” 4 ”	1.37	6.00	338

In some experiments, the effect of peptone caused an even greater increase, in others it produced a much smaller effect, but the rate of fermentation was always increased (Table II). This observation means that the rate of acid-formation is controlled not only by glucose, but also by other substances in the medium surrounding the cells. This fact helps to explain the poor growth of bacteria in synthetic media. They have not only a very limited choice of building material, but their rate of fermentation, and consequently their vital energy, is considerably lower than in the presence of peptone.

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[1] Rahn, O., Hegarty, C. P., and Deuel, R. E., *J. Bact.*, 1938, **35**, in press.

Two explanations for this additional effect of peptone present themselves. Either some part of the peptone is an accessory fermentation-factor, perhaps of the nature of a co-zymase, or the peptone contains a reducing compound which keeps out the oxygen, or establishes a redox potential more favorable for lactic-acid formation.

Previous experiments (loc. cit.) had shown an air-current to retard, and a nitrogen-current to accelerate the rate of fermentation compared with that of a suspension standing quietly in air. If peptone acted merely as oxygen-acceptor, it should have no effect in an atmosphere of nitrogen. However, experiments showed the increase in nitrogen to be larger than in an air-current.

Na_2SO_3 was used to reduce the redox potential.[2] In all experiments, with or without peptone, with amounts varying from 0.05 to 0.40% (anhydrous), fermentation was retarded by this reducing compound. Thioglycolic acid retarded slightly, and so did iron powder reduced in hydrogen. On the other hand, the oxidizing compound $K_3Fe(CN)_6$, in concentration of 0.16%, accelerated fermentation in presence of peptone, and was without influence when peptone was omitted. It does not seem probable, from these results, that the peptone compounds or other stimulating agents act by establishing a more favorable redox potential. The alternative is the assumption of an accessory fermentation-factor.

Our search for the chemical nature of the active substances in peptone was started by testing the compounds which according to Meyerhof and Lohmann, Warburg, and von Euler[3] are contained in the co-zymase system. Since we worked with living cells, it seemed sufficient to supply the simple components, nicotinic acid and adenine. Ribose as such could not be tested on account of the prohibitive price. The small amount furnished in 500γ lactoflavin per 100 cc. produced no effect. Adenine in quantities of 0.02 to 0.60% produced no effect except when in combination with several other compounds. Nicotinic acid (0.002%) stimulated the rate of fermentation in every experiment (Table II).

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[2] Knaysi, G., and Dutky, S. R., *J. Bact.*, 1934, **27**, 109.

[3] A summary by Robison of the very extensive literature may be found in *Annual Review of Biochemistry*, 1936, **5**, 187.

Another substance important for the production of lactic acid in muscle gave positive results with bacteria, namely magnesium ions. Concentrations of 0.05 or 0.10% $MgSO_4$ were used; 0.01% did not seem quite sufficient for maximal rates. In all later experiments, $MgSO_4$ was added to the buffer. Since the glucose buffer is free from nitrogenous substances, ammonium chloride, asparagin, alanine and cysteine were added. Only the latter gave a slight increase when 0.1% was offered. Inositol, a compound important for yeast growth, had no effect. Lactoflavin, which had been found by Orla-Jensen[4] to increase the final amount of acid in cultures of various lactic-acid bacteria, showed no change in the rate of fermentation when 500γ per 100 cc. were added.

Most of our experiments centered around the variable effect of ascorbic acid. This was always used in a concentration of 0.02%. A comparison of all experiments showed that vigorous fresh cells were not benefited by ascorbic acid, in fact, there was a decrease in some cases. When the cells were injured, by heating, by holding at 0°C. for several days, or by the use of water in place of buffer, the power of cells to produce colonies on agar was lost more rapidly than their fermenting capacity. The same has been observed before with yeast cells by Rahn and Barnes.[5] In all these cases, the cells were benefited by ascorbic acid (Table II).

In 3 experiments, the centrifuge became so overheated that the cells failed altogether to produce colonies, though they still fermented. Here, the ascorbic acid caused the greatest increase, namely, 400%, 500% and 1100%. Different kinds of injury all resulted in a need for ascorbic acid, and it seems that the greater the injury, the greater is the benefit from this compound, relatively as well as absolutely, in mg. of acid per equal quantities of cells. This may be seen from the table. It is also evident when comparing the effect of the holding-time at 0°C. in different experiments. The increase obtained from addition of ascorbic acid was

	%
with cells tested fresh	– 3
" " held ½ day at 0°	–16
" " " 1½ days " "	7
" " " 2 " " "	63
" " " 3 " " "	30

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[4] Orla-Jensen, S., Otte, N. C., and Snog-Kjaer, A., *Mémoires Acad. Sciences Danemark*, 9th ser., 1936, **6**, 5.

[5] Rahn, O., and Barnes, M. N., *J. Gen. Physiol.*, 1932, **16**, 579.

TABLE II.

Increase of Rate of Lactic Acid Formation by Peptone, Nicotinic and Ascorbic Acids.

Cell treatment	Million cells per cc.	F.C. in buffer, 10^{-10} mg.	Percentage increase by addition of .5% peptone	.002% nicotinic acid	.02% ascorbic acid	nicotinic plus ascorbic acids
I. Washed twice	4,900	1.54	94	—	9	115
II. a ,, in buffer, stored in buffer	5,000	2.52	60	—	24	—
b ,, ,, ,, ,, as moist cells	3,800	2.52	100	—	25	—
c ,, ,, water, ,, in buffer	2,000	3.15	120	—	85	—
d ,, ,, ,, ,, ,, water	1,780	3.51	100	—	75	—
e ,, ,, ,, ,, as moist cells	1,120	4.80	140	—	116	—
III. a Cells washed twice	2,200	4.46	70	31	–21	35
b Same cells, heated 20 min. to 42°C	1,500	4.10	130	52	0	52
c ,, ,, ,, 10 ,, ,, 45°C	1,800	4.40	110	56	– 5	59
d ,, ,, ,, 20 ,, ,, 45°C	1,215	4.40	120	75	20	75
IV. a Cells washed twice, tested at once	3,700	4.22	77	15	3	67
b Same cells, heated in centrifuge to 42°	3,250	3.88	76	22	14	63
c ,, ,, ,, ,, ,, ,, 45°	2,080	2.37	120	46	66	135
V. a Cells washed twice	2,600	2.42	176	58	64	—
b Same cells heated in centrifuge to 40°C	1,400	4.28	102	30	60	—

Combinations of ascorbic and nicotinic acid are often, but not always, additive. The combined effect is rarely as high as that of peptone. It is of interest to note that in the presence of ascorbic plus nicotinic acid, adenine and cysteine increase the rate while they show little or no effect when alone. This seems to be a simple case of limiting factors.

The increased fermentation by injured cells makes it seem probable that ascorbic acid is a regular constituent of all cells, but is not needed by normal cells because they can supply their own demand. The present results do not indicate whether the injury is due to increased permeability of the membranes, to autolysis of important constituents, or to other causes. After injury, fermentation depends, at least partly, upon the ascorbic acid diffusing into the cells from the surrounding medium. Peptone and nicotinic acid do not show this increased effect with injured cells.

Summary. The formation of lactic acid by washed cells of *Streptococcus lactis* is increased by the presence of ascorbic acid or nicotinic acid. The two compounds act differently. Ascorbic acid stimulates mostly (perhaps exclusively) injured or exhausted cells while nicotinic acid affects normal and injured cells equally. The combined action of the two acids is usually, but not always additive, and approximates the effect of peptone. When both nicotinic and ascorbic acid are present, other compounds like cysteine or adenine may increase the rate of fermentation while these compounds alone have no such effect.

9798

Nature of the Reticulocytosis in Pernicious Anemia Following Liver Therapy.

OLIVER P. JONES. (Introduced by W. J. Atwell.)

From the Department of Anatomy, University of Buffalo, and the Hematological Laboratory, Department of Anatomy, University of Minnesota.

Since the original publications by Minot, *et al.*,[1] it has been constantly observed that, in uncomplicated cases, pernicious anemia patients during relapse exhibit a reticulocytosis in the peripheral blood following the administration of liver extract which is, in general, inversely proportional to the degree of anemia. This has been interpreted to indicate a reactivation of megaloblasts to mature to later stages of normal erythropoiesis.[2] Moreover, Doan[2a] has considered it quite identical, both qualitatively and quantitatively, with that found in hypochromic anemias after iron therapy. These interpretations have been based upon Doan, Cunningham and Sabin's theory[3] that megaloblasts are the earliest recognizable stage in normal erythropoiesis. Consequently, this has given rise to the concept advocated by Peabody, Doan and Isaacs[4] that the marrow lesion in pernicious anemia is a hyperplasia of cells at the megaloblast level which have failed to mature to later stages and elaborate hemoglobin.

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[1] *a.* Minot, G. R., Murphy, W. P., and Stetson, R. P., *Am. J. Med. Sci.*, 1928, **175**, 581; *b.* Minot, G. R., Cohn, E. J., Murphy, W. P., and Lawson, H. A., *ibid.*, 1928, **175**, 599.

Contrary to these opinions, there is an ever-increasing amount of evidence that genuine pernicious anemia megaloblasts are not present in normal bone marrow and are not related to normal erythropoiesis.[5] Also, in pernicious anemia, there is not a failure of cells to differentiate beyond a certain level of maturation but rather, the development of a pathologic red cell series. The ultimate products of this series are reticulated and mature megalocytes.[5a, 6] The latter is the most obvious answer to the query raised by Persons.[7] Furthermore, Kirschbaum[8] has shown that pernicious anemia megaloblasts are similar to the primitive erythroblasts of the embryonic yolk-sac but not identical with them.

The present investigation is concerned chiefly with a study of pernicious anemia bone marrows before, during and at the peak of the reticulocyte response following the administration of massive doses of liver extract. Material for this investigation consisted of 20 sternal marrows obtained before therapy and 25 secured from 10 hours to 18 days after therapy.* All dry smears and imprints were stained with May-Grünwald-Giemsa combination of Pappenheim. Reticulocytes were studied in preparations made by smearing freshly aspirated sternal marrows upon a slide previously treated with an alcoholic solution of brilliant cresyl blue. Smears thus prepared were allowed to remain in a moist chamber for 5 minutes and then dried rapidly. Some of these preparations were counterstained. At least one thousand cells were examined in each marrow.

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[2] *a.* Doan, C. A., *Medicine,* 1931, **10**, 323; *b.* Isaacs, R., *Phys. Rev.*, 1937, **17**, 291; *c.* Osgood, E. E., and Ashworth, C. M., *Atlas of Hematology*, J. W. Stacey, Inc., San Francisco, 1937; *d.* Kracke, R. R., and Garver, H. E., *Diseases of the Blood and Atlas of Hematology*, J. B. Lippincott Co., Phila., 1937.

[3] Doan, C. A., Cunningham, R. S., and Sabin, F. R., *Carnegie Contributions to Embryology*, 1925, **16**, 163.

[4] *a.* Peabody, F. W., *Am. J. Path.*, 1927, **3**, 179; *b.* Doan, C. A., *J. Lab. and Clin. Med.*, 1932, **17**, 887; *c.* Isaacs, R., *Am. J. Med. Sci.*, 1937, **193**, 181.

[5] *a.* Jones, O. P., *Handbook of Hematology* (Ed. by Hal Downey), Paul Hoeber, N. Y., in press; *b.* Dameshek, W., and Valentine, E. H., *Arch. Path.*, 1937, **23**, 159; *c.* Rohr, K., *Knochenmarksmorphologie des menschlichen Sternal punktates.*, Urban and Schwarzenberg, Berlin, 1937; *d.* Schulten, H., *Die Sternalpunktion als Diagnostiche Methode*, G. Thieme, Leipzig, 1937; *e.* Segerdahl, E., *Über Sternalpunktionen*, Appelbergs, Upsala, 1935; *f.* Nordenson, N. G., *Studies on Bone Marrow from Sternal Puncture*, Börtzells, Stockholm, 1935; *g.* Mallarmé, J., *Le Myélogramme*, G. Doin & Cie, Paris, 1937.

[6] Schartum-Hansen, H., *Folia Hematol.*, 1937, **58**, 145.

[7] Persons, E. L., *J. Clin. Invest.*, 1929, **7**, 615. See top of p. 628.

[8] Kirschbaum, A., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 542.

During relapse, megaloblasts constitute from one-fifth to one-half the total marrow cells. Our averages show that promegaloblasts and basophilic megaloblasts form 6% of the total cells while polychromatic and orthochromatic megaloblasts constitute 24%. All transitional stages exist between the latter and reticulated and mature megalocytes. Cells of the normal series, which are in a quiescent state, constitute about 3%. We have recently shown that in addition to the development of a pathologic red cell series there is also a pathologic regeneration of the neutrophils.[9]

After liver therapy, normal erythropoiesis is reëstablished by heteroplastic development from the myeloblast and by homoplastic development of the dormant normoblastic series. During this activity there is not a wholesale transformation of the megaloblastic series into normoblasts, but rather, megaloblasts complete their maturation as reticulated and mature megalocytes.[10, 11, 12] The fate of megaloblasts after liver therapy parallels that of the primitive erythroblasts of the embryo following the appearance of the liver anlage, in that they complete their maturation and finally die out.[10, 12, 13] Naegeli[12] has reported megaloblasts disappear from the marrow 8 days after therapy and believes that it is a gross error to assume that they have matured to normal cells. In general, our material supports this view, but in some cases orthochromatic megaloblasts were present 18 days after therapy. In a few instances, 8 to 12 days after therapy, hemoglobin-bearing megaloblasts appeared to be undergoing a peculiar granular degeneration while in the marrow.

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* This material was made available by the generous cooperation received from Dr. C. J. Watson, Dr. I. J. Pass, and other members of the staff of the Department of Medicine, University of Minnesota Medical School.

[9] Jones, O. P., *Arch. Int. Med.*, 1937, **60**, 1002.

[10] Schwarz, E., *Wien. klin. Wchnschr.*, 1928, **41**, 192.

[11] Rohr, K., *Praxis, Schweiz. Rdsch. Med.*, 1935, No. 26.

[12] Naegeli, O., *Wien klin. Wchnschr.*, 1935, **48**, 225.

[13] Kirschbaum, A., and Downey, H., *Anat. Rec.*, 1937, **68**, 227. See figs. 3-6.

TABLE I.

Patient	Days After Liver	Blood Reticulocyte %	Marrow Erythroblasts % of all cells		
			Normal Series	Polychrom. Megaloblasts	Orthochrom. Megaloblasts
W.M.	4	26	33.0	—	3.5
W.K.	4	22	33.6	2.4	8.5
J.B.	4	18	40.5	1.0	3.8
P.L.	5	17	25.0	1.3	8.2
M.H.	5	18.8	44.9	0.5	4.9
B.P.	4 (peak)	21	43.8	1.3	2.9

Table I shows that during the rise of reticulocytes in the blood, there are still cells in later stages of megaloblastic development in the marrow which have not yet completed their differentiation to reticulated and mature megalocytes. All intermediate stages between the latter and the remaining megaloblasts are present. It is obvious, therefore, that the total reticulocyte population is composed of cells derived from 2 entirely different sources, *i. e.*, the pathologic series and the normal series. Hence, the reticulocyte response after liver therapy in pernicious anemia not only indicates a renewed activity on the part of normal erythropoiesis but it also indicates that the marrow is being purged, so to speak, of its pathologic red cell series, the megaloblasts. In all probability, this purging effect of liver extract upon the bone marrow explains the rise in the mean reticulocyte diameter as well as the subsequent decrease to normal values which were observed by Fitzhugh and Persons.[14] It has also been reported that a few reticulated megalocytes remain in the blood until the erythrocyte count has reached between 2 and 3 million.[15]

This interpretation of the dual nature of the reticulocytosis is in agreement with certain mathematical formulæ[16] devised to predict the height of a response from the initial erythrocyte count. Furthermore, it can now be more fully appreciated why the reticulocytosis should be inversely proportional to the degree of anemia. For, in general, the more severe the anemia the more hyperplastic[4a] and more megaloblastic[6] the marrow. Accordingly, there would be more cells of the pathologic series available to be transformed into reticulated megalocytes for the response, in addition to the normal reticulocytes. For practical purposes discrimination of the two reticulocyte types is unnecessary.

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[14] Fitzhugh, G., and Persons, E. L., *J. Clin. Invest.*, 1929, **7**, 631.

[15] Davidson, L. S. P., and McCrie, J. G., *Lancet*, 1928, **215**, 1014.

[16] $R = \frac{0.73 - 0.2\,E_0}{0.73 + 0.8\,E_0}$; Riddle, M. C., *Arch. Int. Med.*, 1930, **46**, 417.

Wintrobe[17] has shown that the increased mean corpuscular volume during liver therapy is due to an outpouring of reticulocytes from the marrow. This marked increase in corpuscular volume can also be more fully appreciated when it is considered that part of the cells are reticulated megalocytes which are larger than normal reticulocytes.[15]

The participation of reticulated megalocytes in the reticulocytosis of pernicious anemia patients following liver therapy makes this response qualitatively distinct and different from that in hypochromic anemias after iron therapy, since it is well established that marrows from the latter do not possess megaloblasts. In like manner, the reticulocyte response in guinea pigs after the administration of liver extract[18] is qualitatively different from that in pernicious anemia patients since these animals do not have megaloblasts in their marrow.[19] Reports to the contrary by Gall[20] are due to the fact that he has adopted Sabin's definition of the term megaloblast which indiscriminately includes primitive erythroblasts, promegaloblasts and pronormoblasts (proerythroblasts).

Conclusion. After liver therapy megaloblasts complete their maturation as reticulated and mature megalocytes. The resultant reticulocytosis is composed of reticulated megalocytes and normal reticulocytes. This not only indicates the reëstablishment of normal erythropoiesis but also, that the marrow is being purged of its pathologic red cell series, the megaloblasts. This response is qualitatively distinct from the reticulocytosis of hypochromic anemias following iron therapy and the one in normal laboratory animals after liver treatment.

9799 P

Gonadotropic Hormones in the Hereditary Dwarf Mouse.

ALFRED MARSHAK. (Introduced by Shields Warren.)

From the Laboratory of Pathology, New England Deaconess Hospital, Boston.

Snell[1] described dwarfism in the mouse which was inherited as a recessive character. Smith and MacDowell[2] later showed that, in these mice, the eosinophiles were missing from the anterior lobe of the pituitary and that the growth hormone was either not present at all, or else was produced in amounts much less than in the normal. Transplantation experiments[3] were interpreted as indicating that pituitaries of the dwarfs contained a higher concentration of gonadotropic hormone than the normal. Nevertheless, sexual development was markedly retarded in both male and female, copulation and reproduction being impossible unless normal pituitary lobe was supplied by implantation or injection. Thus, it was difficult to understand why the gonadotropic hormone of the dwarf did not give the proper stimulus to its own gonads and accessory sex glands unless a synergistic action of the growth hormone was involved. These experiments were performed with relatively young mice and another interpretation seems possible from observations on older dwarfs which are to be described below.

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[17] Wintrobe, M. M., *J. Clin. Invest.*, 1934, **13**, 669.

[18] Jacobson, B. M., *J. Clin. Invest.*, 1935, **14**, 664.

[19] Jones, O. P., *J. Lab. and Clin. Med.*, 1936, **21**, 335.

[20] Gall, E. A., *Am. J. Path.*, 1937, **13**, 575.

[1] Snell, G. D., *Proc. Nat. Acad. Sci.*, 1929, **15**, 733.

Heterozygous parents were obtained from Snell in 1933 and dwarfs bred from these. In addition 2 dwarf males and 6 dwarf females were obtained from MacDowell in 1936. In all of 6 males observed after they had reached an age of a year or more priapism had developed. One such animal was given 2 injections of a suspension of beef anterior lobe at 2-day intervals and the priapism disappeared within a week after the first injection. In 2 animals which were kept untreated for 17 months, the priapism was maintained constantly for the last 6 months. These untreated dwarfs were then sacrificed and were found to have very strikingly enlarged seminal vesicles. Microscopic examination showed that the enlargement was due to retained secretion. In spite of very marked distension of the glands, their epithelial lining appeared to be of the normal columnar type and showed the usual secretion granules. There was little or no difference between normal and dwarf mice in the number of interstitial cells of the testis or the amount of spermatozoa in the testis and epididymis. In the dwarf the vas deferens contained sperm and had an apparently normal epithelium; the urethra was filled with semen containing occasional spermatozoa.

Female dwarfs of the same age had several follicles in each ovary. However, these became atretic before there was any very marked antrum development.

The experiments of Greep and Fevold[4] show that the luteinizing hormone of the pituitary (LH) when injected into hypophysectomized males will produce pronounced enlargement of the seminal vesicles and will stimulate, probably indirectly, gametogenesis; but the follicle stimulating hormone (FSH) will not induce any enlargement of the seminal vesicles. In the female Foster and Hisaw[5] have shown that FSH will stimulate the development of follicles only after they have developed antra. Epithelioid cells atrophy after hypophysectomy and enlarge again after injections of LH.[6] Thus, if the above mentioned conclusions be accepted, the enlarged seminal vesicles and the correction of priapism with anterior lobe injections in the male dwarf mouse may be taken to indicate the presence of LH and a partial or complete deficiency of FSH; and in the female, likewise, the presence of large numbers of epithelioid cells and the development of follicles only to medium size again indicate the presence of LH and a deficiency of FSH. It seems probable, therefore, that the dwarf mouse not only lacks growth hormone, but has a partial or complete deficiency of at least one of the gonadotropic hormones.

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[2] Smith, P. E., and MacDowell, E. C., *Anat. Rec.*, 1930, **46**, 249.

[3] Smith, P. E., and MacDowell, E. C., *Anat. Rec.*, 1931, **50**, 85.

[4] Greep, K. O., and Fevold, H. W., *Endocrinol.*, 1937, **21**, 611.

9800

Method for Separation of Micro-organisms from Large Quantities of Broth Culture.

JONAS E. SALK. (Introduced by R. Keith Cannan.)

From the Department of Chemistry and the Department of Medicine, New York University College of Medicine.

This paper contains a report of a rapid and simple method for removing bacteria from broth cultures. Large volumes can be handled conveniently. Preliminary tests indicate that the antigenic properties of the bacteria remain unaffected.

The method consists in the formation of a precipitate of calcium phosphate in the broth. This adsorbs the bacteria, settles quickly, and can be removed by siphoning away the supernatant liquid and lightly centrifuging the remaining sediment. The calcium phosphate may then be redissolved by suspending the precipitate in water and acidifying to about pH 5.0. The bacteria can be recovered by centrifugation and then washed.

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[5] Foster, M. A., Foster, R. C., and Hisaw, F. L., *Endocrinol.*, 1937, **21**, 249.

[6] Hisaw, F. L., personal communication.

The amounts of bacterial culture employed are limited only by the available apparatus. A preliminary test is made of the amount of calcium phosphate necessary to effect practically complete removal of the microörganisms. This may be done by preparing a series of tubes containing 10 cc. of broth to which is added 0.1-1.0 cc. of the calcium reagent, followed by N NaOH, to pH 7.0. After light centrifugation, the supernatant liquid will be crystal-clear in the tubes containing enough calcium. In the routine treatment of any one type of culture, a single test should suffice.

We conduct the precipitation of 5-liter quantities of culture by adding half the required amount of 0.5 M acid calcium phosphate,* and half the amount of N NaOH that will be required to bring the pH to 6.5-7.0 (Nitrazine paper). The rest of the calcium phosphate and NaOH are then added. This is probably more efficient than adding the whole amount at one time, and helps to prevent large clumps of precipitate from forming. For the same reason, the mixture should be agitated vigorously during the precipitation. There is little danger of excessive local hydroxyl ion concentration because of the buffer action of the protein and peptone present in the broth.

The precipitate is allowed to settle for 10 minutes or more and the supernatant liquid, if not turbid, siphoned off and discarded. The amount of bacteria lost in this way is negligible. The precipitate is then separated by light centrifugation for 2-3 minutes or suction filtration. Should the supernatant liquid be turbid, it is well to determine whether this is due to the calcium phosphate or to bacteria. If due to the former, it will clear readily with a little acid, and flocculate with a little alkali. If the supernatant liquid does not become crystal clear after acidification, insufficient calcium phosphate was used and the treatment should be repeated.

The precipitate of calcium phosphate and bacteria should be mixed with water (so that the final volume is half the volume of the calcium reagent used) to form a homogeneous suspension. One or 2 N HCl is added, with constant stirring, to a pH between 5 and 4. The calcium phosphate precipitate dissolves, and the bacteria may be collected with the aid of centrifugation. If it is desired to restrict the acidity of the bacterial suspension as much as possible, the calcium phosphate may be redissolved at about pH 5 if sufficient water is used, or the acidity may be controlled more safely by adding HCl to pH 5.5, followed by enough normal acid sodium phosphate to complete the solution.

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* To prepare a $Ca(H_2PO_4)_2$ solution in 0.5 M concentration, acidification with HCl to pH 3.5-3.0 is necessary to dissolve the salt completely.

In the case of a culture containing a phosphate buffer, it is only necessary to add an adequate amount of calcium chloride, followed by neutralization with NaOH, in the original precipitation.

We found that a culture of *Staphylococcus aureus* and one of *Bacillus typhosus* grown in beef-heart infusion-broth† required approximately 0.014 Mols of acid calcium phosphate per liter, for practically complete precipitation. A glucose phosphate broth culture‡ of indifferent streptococcus (Strain Q155) required 0.04 Mols of $CaCl_2$ per liter.

We have tested some of the antigenic properties of the Q155 Strain of indifferent streptococcus and found that the sera of rabbits, immunized with a vaccine prepared from bacteria precipitated by this method, when compared with the sera of rabbits immunized with a vaccine prepared from centrifuged organisms, contained antibodies (type-specific agglutinins and precipitins) detectable in equally high dilutions.

Furthermore, crude extracts containing the type-specific carbohydrate (S)[1] were prepared, both from precipitated and centrifuged bacteria, and they were found to be active in the same dilutions. The extracts were prepared according to Lancefield's method.[2]

Quantitative tests of viability have not been made, but we can say that the majority of bacteria is viable after separation by this method.

We find that less time is required to separate bacteria from 20 liters of broth, by this method, than was formerly necessary to handle 3 liters; and larger quantities require very little additional time. Furthermore, the yield of bacteria is greater than after the usual centrifugation procedure.

We are investigating the possibility of using other salts as precipitating agents, and the effect of these procedures on labile antigenic fractions of some bacteria.

The writer wishes to acknowledge the kind interest and advice of Drs. R. K. Cannan and Currier McEwen.

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† Beef heart infusion broth: beef heart infusion; 1% peptone; 0.5% NaCl.

‡ Glucose phosphate broth: beef heart infusion; 1% peptone; 0.2% Na_2HPO_4; 0.5% glucose; adjusted to pH 7.6-7.8 (N NaOH).

[1] Hitchcock, C. H., *J. Exp. Med.*, 1928, **48**, 393.

[2] Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

9801 P

Effect of Choline on Production of Experimental Atherosclerosis in Rabbits.

ALFRED STEINER. (Introduced by Kenneth B. Turner.)

From the Research Division of Chronic Diseases, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University.

Best and his coworkers[1, 2, 3] have shown that the administration of choline prevents the fatty infiltration of the liver in rats fed fat or cholesterol. The present study was designed to determine whether choline had any effect on the deposition of cholesterol esters in the arteries of rabbits fed cholesterol.

Methods. Thirty-eight male, chinchilla rabbits approximately 6 months old were used. The animals were kept indoors in individual cages. The diet consisted of oats and fresh vegetables. One gram of cholesterol was mixed into the food of each rabbit 3 times a week.

The animals were divided equally into 2 groups, 19 in each. Group I consisted of control rabbits fed only cholesterol. Group II rabbits were given choline in addition to cholesterol. Seventeen of the second group were fed 500 mg. of choline daily and the remaining two 750 mg. daily. The choline was administered by dissolving it in 5 cc. of water and then mixing it with a combination of oats and ground carrots. Food was withheld from the animals for 5 hours before the feeding of cholesterol and choline in order to insure ingestion.

Blood was obtained from the ear vein at weekly or bi-weekly intervals. Cholesterol determinations were made on the whole blood by the method of Bloor, Pelkan and Allen.[4]

The rabbits died or were killed over a period of from 40 to 100 days after the beginning of the experiment. An autopsy was done in each case and the extent of fatty change in the aorta, liver, and kidneys as shown macroscopically, and the degree of enlargement of the adrenals, was noted and graded from zero to 4 plus.

Level of blood cholesterol. All except 3 of the animals in both groups exhibited an immediate, progressive and sustained rise in the level of the blood cholesterol. In rabbits Nos. 126, 140, and 145, the amount of cholesterol in the blood was so great that it crystal-

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[1] Best, C. H., and Huntsman, M. E., *J. Physiol.*, 1932, **75**, 405.

[2] Best, C. H., Ferguson, G. C., and Hershey, J. M., *Ibid.*, 1933, **79**, 94.

[3] Best, C. H., and Ridout, H. H., *Ibid.*, 1936, **86**, 343.

[4] Bloor, W. R., Pelkan, K. F., and Allen, D. H., *J. Biol. Chem.*, 1922, **52**, 191.

lized out of the alcohol-ether solution after standing for a short time in the ice box. The animals that did not show a significant increase in the level of blood cholesterol were Nos. 150, 159, and 162 of Group I. It is known that some rabbits, usually males, are "resistant" to cholesterol feeding and do not develop either hypercholesterolemia or lipoid infiltration into the tissues.

There was no appreciable difference in the levels of blood cholesterol between Group I, the control, and Group II, the choline-fed animals.

Gross appearance of the tissues. Autopsies were done on the rabbits at varying intervals from 40 to 100 days after the beginning of the feeding. In Group I, 15 showed definite gross atheromatous plaques, 16 lipoid deposits in the liver, 10 enlargement of the adrenals, and 13 deposition of lipoid in the kidneys. In Group II, 9 showed definite gross atheromatous plaques, 16 gross lipoid infiltration of the liver, 5 adrenal and 8 renal lipoid infiltration. The autopsy results show a greater amount of lipoid involvement in the control rabbits. On a statistical basis the results are not striking.

TABLE I.
The Occurrence of Gross Aortic Atheromata in Groups I and II.

Days	40	45	50	60	65	70	80	90	100
Group I									0
Rabbits fed	—	+	0	++	++	+++		+	++
cholesterol		+		+		+		++	0
				++		++++	+++		++
									+
									0
									+++
Group II		0				0		+	+
Rabbits fed	0	0	0	0	0	0	0	+	+
cholesterol									+
and choline									+++
									+
									++
									++

However, Table I reveals a definite and significant difference between the autopsy findings of the 2 groups in those animals killed from the 40th through the 80th day of the experiment. In Group II, the rabbits fed choline, 10 were killed between the 40th and 80th day, and none showed macroscopic lipoid infiltration of the aorta. In Group I, 11 animals were killed during this same period on corresponding days and 10 of these exhibited characteristic gross atherosclerosis.

The extent of lipoid infiltration in the liver, adrenal and kidney was likewise less in Group II, but the difference was not as great as in the aorta.

All of the animals killed after the 80th day showed gross lipoid involvement of the aorta except numbers 150, 159, and 162 of Group I. These 3 rabbits whose blood cholesterol did not rise significantly, were considered to be "resistant".

The results reported by Best and his coworkers quoted above were not obtained in the choline-fed rabbits. Gross lipoid deposition in the liver occurred in 16 out of 19 animals.

Summary and Conclusions. 1. The oral administration of 0.5 gm. of choline daily to 19 rabbits fed 1 gm. of cholesterol 3 times weekly did not prevent the development of a hypercholesterolemia comparable to that produced in 19 control animals fed cholesterol alone. 2. Ten of the choline-fed rabbits killed at intervals between the 40th and 80th days failed to show macroscopic atheromata in the aorta. Ten of the 11 control rabbits fed the same amounts of cholesterol and sacrificed at similar periods showed gross atherosclerosis. 3. After 80 days the macroscopic lesions of the aorta were similar in both groups. 4. It is concluded that choline delays but does not prevent atherosclerosis in cholesterol-fed rabbits.

9802 P

Magnesium as a Bronchodilator Agent in Perfused Guinea Pig Lungs.

VICTOR G. HAURY. (Introduced by Charles M. Gruber.)
From the Department of Pharmacology, Jefferson Medical College, Philadelphia.

A series of perfusion experiments were performed on isolated guinea pig lungs to test the efficacy of magnesium as an antagonist against the bronchial constriction produced by histamine, pilocarpine and barium chloride.

Isolated guinea pig lungs were used. Except for slight modifications, the procedure was essentially the same as that described by Sollmann and Von Oettingen[1] and Tainter, Pedden and James.[2] Histamine 1:10,000, pilocarpine 1:1000 and barium chloride 2.5% were used as the constrictor drugs. Magnesium sulfate ($MgSO_4.7H_2O$) was used in a 10.13% solution (1 cc. = 10 mg.

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[1] Sollmann, T., and Von Oettingen, W. F., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 692.

[2] Tainter, M. L., Pedden, J. R., and James, M., *J. Pharm. and Exp. Therap.*, 1934, **51**, 371.

Mg.). The antagonistic action of magnesium against histamine and pilocarpine was studied by injecting a mixture of the constrictor drug and magnesium sulfate into the perfusion fluid, and comparing the perfusion rate thus obtained with the perfusion rate obtained when the constrictor drug was injected alone. The antagonistic action of magnesium against barium constriction was studied by adding magnesium sulfate to the perfusion fluid at the time of maximum barium constriction. Tainter's solution[2] was used as the perfusion fluid.

The action of the drugs studied was interpreted quantitatively by measuring the change in perfusion rate in cubic centimeters per minute and comparing this with the previously established control perfusion rate. The results were calculated as percentage increase (dilation) or decrease in perfusion rate (constriction). In 8 experiments on the isolated lung of 5 guinea pigs, 0.6 cc. of 1:10,000 solution of histamine produced 78 to 91% (average 82) constriction. In 5 experiments on the same lungs, 0.6 cc. histamine plus 1.5 cc. of magnesium sulfate produced only 15 to 53% (average 33) constriction. Maximum constriction in both instances occurred in 4 to 5 minutes. Recovery from histamine constriction required an average of 20 minutes while recovery from histamine plus magnesium occurred in 12 minutes.

In the perfused isolated guinea pig lung magnesium sulfate was found to be more antagonistic against pilocarpine constriction than against histamine constriction. In 5 experiments on 5 isolated lungs, 1.0 cc. of a 1:1000 solution of pilocarpine produced 43 to 66% (average 55) constriction. In the same number of experiments 1.0 cc. pilocarpine plus 1.0 cc. magnesium sulfate produced an actual dilation of 7 to 61% (average 27) over the previous control values.

That magnesium is effective against barium constriction was recently shown by Sollmann and Gilbert.[3] In the study reported here 0.35 cc. of barium chloride was found to produce maximum decrease (77%) in perfusion rate in from 2 to 3 minutes with spontaneous recovery in from 22 to 38 minutes (average 30). Magnesium sulfate injected into the perfusate at the point of maximum barium constriction shortened the recovery period by 75%.

Conclusion. Magnesium is an effective antagonist to pilocarpine and barium constriction, and less effective against histamine constriction.

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[3] Sollmann, T., and Gilbert, A. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 16.

9803

Uterine Reaction to Testosterone in the Rat.

J. B. Brooksby. (Introduced by J. B. Collip.)
From the Department of Biochemistry, McGill University, Montreal.

The administration of testosterone to the female animal leads to an enlargement of the uterus, which has been described in the rabbit by Robson[1] and by Klein and Parkes[2] as a progestational proliferation. McKeown and Zuckerman[3] have recently described the reaction of the rat uterus to testosterone, and noted that the effect is not obtainable in ovariectomized animals, the deduction being that the uterine effect is due to increased production of the normal ovarian hormones under the influence of testosterone. The work of Selye and McKeown[4] on the reaction of the uterus to trauma by the formation of deciduoma under the influence of progesterone, or of an endometrial mole under the influence of oestrone and progesterone suggested the use of traumatization as an index of the state of the endometrium, and the following experiments were undertaken to examine the uterine growth produced by testosterone and, if possible, to arrive at the nature of this growth.

In connection with experiments on lactation, testosterone propionate was given in doses of 500 γ in 0.25 cc. oil daily for the first 10 days of lactation to 6 rats. Another group of 6 rats remained untreated and a third group received one mg. of crystalline progesterone daily in 0.25 cc. oil. At the end of 10 days the animals were killed and the uteri examined. Those of the testosterone group were markedly enlarged and hyperemic, showing on histological examination an endometrium resembling the type termed by Selye, Collip and Thomson[5] "second progestational proliferation" with a loose fibrous stroma. The epithelium, however, was lower than would be expected in this type of endometrium and the ridges of epithelium so often seen were not marked. The uteri of the progesterone-treated group were only slightly enlarged and were in the stage of "first progestational proliferation," while the control group differed from the progesterone-treated group only in the smaller size of the uterus.

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[1] Robson, J. M., *Quart. J. Exp. Physiol.*, 1937, **26**, 355.

[2] Klein, M., and Parkes, A. S., *Proc. Roy. Soc. B.*, 1937, **121**, 574.

[3] McKeown, T., and Zuckerman, S., *Proc. Roy. Soc. B.*, 1937, **124**, 362.

[4] Selye, H., and McKeown, T., *Proc. Roy. Soc. B.*, 1935, **119**, 1.

[5] Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinol.*, 1935, **19**, 151.

A group of 6 rats was ovariectomized on the first day of lactation and given 500 γ testosterone daily. Examination of the uteri on the 10th day showed them to be larger than normal and hyperemic. Histologically the uterus was of the dioestrous type with, however, considerable cell proliferation under the epithelium, suggesting that testosterone was capable of inhibiting to some extent the castration regression of the uterus, particularly with regard to the stroma.

The effect of testosterone in conjunction with uterine trauma was investigated in 20 rats, 10 normal and 10 castrate, which received 500 γ daily. After 5 days' treatment, biopsy specimens of the uterus were taken from 5 animals of each group, and the uterus subjected to trauma by slitting of the uterine horns in all 20 animals. After a further 5 to 7 days' treatment with testosterone, the rats were killed and the slit horn of the uterus taken for histological examination. Control animals, 6 normal and 6 castrate, were subjected to the same operative interference but remained untreated. The biopsy specimens after 5 days' testosterone treatment showed the normal animals, testosterone-treated, to have uteri of the type of second progestational proliferation with a lining of tall columnar cells thrown into folds over a loose and mostly fibrous stroma. The ovariectomized treated animals, on the other hand, showed small uteri of the post-castration type with a low cubical epithelium and a stroma of fibrous tissue with no dividing cells.

Examination of the slit uterine horn of both normal and castrate testosterone-treated animals showed absence of either deciduoma or endometrial mole. In normal testosterone-treated animals the uterus was of the same type as at biopsy. The edges of the slit horn were covered by tall columnar epithelium and the stroma under this was markedly proliferated, of loose fibrous character, and showed slight oedema and marked leucocytic infiltration. In the castrate testosterone-treated animals the uteri were similar to the testosterone-treated normals but were much smaller. The epithelium was low, the oedema and leucocytic infiltration of the stroma less marked. The uteri of the intact and castrate controls were small and reacted to the slitting only by loosening of the stroma and infiltration with leucocytes. It would seem that the reaction to slitting the uterine wall is the same in the 4 groups studied, *viz.*, some proliferation and loosening of the stroma with leucocytic infiltration. The larger and more active uterus of the testosterone-treated intact animal reacts more obviously, though in essentially the same way as that of the testosterone-treated castrates or that of the untreated animal.

The absence of alteration by testosterone of the reaction to uterine trauma found in the experiments suggested investigation to determine if testosterone had an inhibiting effect on formation of deciduoma when these would normally occur. Six rats were taken on the first day of lactation and given 500 γ testosterone daily. By the fourth day the litters of 2 of these rats had died, though the mammary gland was found to be well developed and secreting, and these 2 animals were removed from the group. On the fifth day biopsy specimens of the uterus were taken and the uterine horn slit in the remaining 4 rats. The biopsy specimen showed the uterus to be in the state of second progestational proliferation, with a lower epithelium than is usual in this condition. Five days later the rats were killed. Histological examination of the slit uterus showed in 3 cases typical deciduomata and in the fourth case a typical endometrial mole. The animal in which mole formation occurred failed to rear its litter. Seven young were born and in 6 days the number was reduced to 3, and in 7 days to *nil*. It is suggested, though direct proof is lacking, that the animal was passing, at the time trauma was performed, into a state of post-lactation oestrus, and that the oestrin in circulation at this time resulted in formation of the endometrial mole. This chance occurrence affords evidence that, in addition to failing to inhibit deciduoma formation, testosterone does not prevent the formation of an endometrial mole.

Summary. From traumatization experiments on the rat uterus, it is concluded that the action of testosterone* differs qualitatively both from that of progesterone and from that of oestrogens.

9804

Hemolytic Properties of Indol.

ERIC PONDER.

From the Biological Laboratory, Cold Spring Harbor.

Rhoads and his collaborators[1, 2, 3] have recently established that the administration of indol to dogs on a deficient (Goldberger) diet results in a severe anemia which can be cured by adding the lacking factors to the diet, and have further shown that this anemia is the result of a hemolytic process rather than of the lack of a growth factor required for normal marrow activity. Because of these observations, the *in vitro* hemolytic properties of indol are of considerable interest, and these have not hitherto been described.

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* The author is indebted to Dr. Erwin Schwenk of the Schering Corporation for the testosterone used in these experiments.

[1] Rhoads, C. P., and Barker, W. H., *J. Exp. Med.*, 1938, **67**, 267.

[2] Rhoads, C. P., and Miller, D. K., *J. Exp. Med.*, 1938, **67**, 273.

[3] Rhoads, C. P., Barker, W. H., and Miller, D. K., *J. Exp. Med.*, 1938, **67**, 299.

1. Indol is a weak hemolysin, a system containing 1.6 cc. of 1 mM/l. indol in 1% NaCl (this is a virtually saturated solution, and has a pH of 6.3), together with 0.4 cc. of a rabbit red cell suspension with $2.5(10^8)$ cells per cc., showing complete lysis in about 30 min. at 25°C. Half this concentration, however, does not show complete lysis for many hours. The lytic effect on the cells of man and of the dog are very similar. As in the case of most lysins, the lytic effect is strongly inhibited by serum or plasma, and, again as in the case of most lysins, the hemolysis is preceded by a disc-sphere transformation. In a saturated solution of indol, this occurs almost immediately.

2. Indol is a powerful accelerator of lysis by saponin, the value of $(R-1)/c$, which measures the accelerating power, being —0.60; this means that indol is about 24 times as acceleratory as benzene (millimol for millimol), and about as potent as iodobenzene. The acceleration of taurocholate hemolysis is smaller, *viz.*, —0.20, or about 8 times that of benzene. These values are at 25°C.

3. Like the effect of benzene and many of its derivatives, the effect of indol on the red cell membrane seems to be a double one. If the cells are brought into contact with the indol, and rapidly washed free of it, their resistance will be found to be scarcely decreased at all: if allowed to stand for some time in contact with the indol, on the other hand, (*e. g.*, 5 min. in 0.2 mM/l. indol), their resistance will be found to be considerably decreased. Sufficiently long contact with greater concentrations results in lysis by the indol *per se*, a clear indication of a direct effect on the cell membrane. The effect of the accelerator is therefore two-fold. In its presence, red cells immediately become more susceptible to the action of lysins, perhaps because the accelerator forms a new phase at the cell surface, and so alters the partition or rate of concentration of the lysin, and this phase of the action of the accelerator is reversible by washing; with time, however, the accelerator reacts irreversibly with the components of the cell surface, even to the point of producing lysis if the concentration is great enough, and the time long enough.

4. Indican, a conjugated form of indol, is neither lytic nor acceleratory; in fact, it is an inhibitor of saponin hemolysis. From a physico-chemical standpoint, however, the water-soluble indican

is very different from the comparatively insoluble indol, and so this result is not unexpected.

These results have a considerable bearing on the way in which the lytic process observed by Rhoads and his coworkers may be brought about, for the flooding with indol of the blood stream of a dog on a deficient diet is equivalent to the flooding of the blood stream with a weak lysin, or, perhaps more significantly, with a powerful accelerator for whatever intravascular lysins may be present. It is true, of course, that the *in vivo* effect of indol will be much less than the *in vitro* effects, partly because no great concentration of indol can occur in the blood stream, and partly because of the great inhibitory effects of the serum proteins and the great number of red cells present. The kinetics of *in vivo* lysis, acceleration, and inhibition, are at present completely unknown, so it is impossible to say exactly what the effect of establishing a *continually maintained* concentration of indol in the blood stream would be; it is safe to say, however, that the result of continually maintaining an accelerator, even in small amounts, in the blood stream would be to hasten any normal lytic process occurring there, and would, therefore, in the long run, tend to bring about an anemia.

9605

Ultracentrifugal Isolation of High Molecular Weight Proteins from Broad Bean and Pea Plants.

HUBERT S. LORING, H. T. OSBORN AND RALPH W. G. WYCKOFF.*
From the Rockefeller Institute for Medical Research, Princeton, New Jersey.

Characteristic high molecular weight proteins have been isolated by ultracentrifugation from the juices of plants diseased with several of the less stable viruses.[1] Various lines of evidence indicate that virus activity is a property of these high molecular weight proteins. The viruses thus far studied are easily transmitted by mechanical means. Certain viruses, however, require specific insect vectors and are difficult to transmit by ordinary mechanical methods. We have made an ultracentrifugal examination of infectious juice containing such a virus, choosing for the purpose pea mosaic (pea virus 1).[2] In nature the pea and potato aphids[2] carry this disease but under favorable conditions it may be transmitted mechanically to about 75% of inoculated plants.

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* Now at Lederle Laboratories, Inc., Pearl River, New York.

[1] Stanley, W. M., and Wyckoff, R. W. G., *Science*, 1937, **85**, 181; Loring, H. S., and Wyckoff, R. W. G., *J. Biol. Chem.*, 1937, **121**, 225.

When the juice from infected broad bean plants, *Vicia faba* L., was ultracentrifuged[3] in a field of 40,000 g. for one and a half hours, appreciable amounts of a heavy material were obtained. The pellets were 2 or 3 times the size of those found in the case of the latent mosaic virus. After 3 ultracentrifugations, a solution of the pellets in water gave the usual qualitative tests for protein and the kind of absorption diagram in the analytical ultracentrifuge that is typical of purified heavy proteins. Nearly all the light "unsedimentable" proteins had been eliminated, and there were present sharp boundaries characterized by the sedimentation constants $s_{20} = 76 \times 10^{-13}$ cm. sec^{-1} dynes^{-1} and $s_{20} = 112 \times 10^{-13}$. Certain samples, in which there seemed to be some decomposition, gave a third boundary with $s_{20} = 54 \times 10^{-13}$.

Activity measurements were made on the juice before ultracentrifugation, on the supernatant liquid after ultracentrifugation, and on the solution of the sedimented pellets. In one experiment in which 20 young broad bean plants were inoculated with the filtered juice before centrifugation, 20 with the supernatant liquid, and 20 with the solution of the pellets obtained after one ultracentrifugation, 6 of the first group, 4 of the second, and 11 of the third showed pea mosaic disease. In a second test, after 3 ultracentrifugations the supernatant liquid failed to infect any of 10 plants, while the solution of the pellets caused infection in 4 of 10 inoculated plants. Though there was in no case a high percentage of infection, the results demonstrated some concentration of the virus in the sedimented pellets. The low specific activity of the sedimented protein indicated that it was not a pure virus protein. This was confirmed when ultracentrifugation of the juice from healthy broad bean plants yielded a similar non-infectious heavy protein. The sedimentation constants measured from this healthy protein solution were the same, within the limits of experimental error, as those from the infectious solution. It is thus apparent that either the concentration of the virus principle in the final solutions was too low to permit detection with the analytical ultracentrifuge or the virus had the same sedimentation constant as one of the normal constituents.

This demonstration of heavy proteins in healthy broad bean plants suggested the examination of another legume. After 3 ultracentrifugations a purified protein giving sharp boundaries with $s_{20} = 77 \times 10^{-13}$, $s_{20} = 117 \times 10^{-13}$ and sometimes $s_{20} = 54 \times 10^{-13}$ was obtained from the juice of healthy pea plants, *Pisum sativum* L. var. *arvense* Poir. It gave the usual protein color tests, contained a pentose, and was probably a nucleo-protein. Both the broad bean and pea plant proteins were pigmented and of limited stability. Solutions of the former were dark green; after repeated sedimentations in the cold the pigment became insoluble leaving a colorless opalescent suspension of inhomogeneous material. Solutions of the pea protein were light green in color and, after standing for several days in the refrigerator, they too became inhomogeneous and no longer sedimented with sharp boundaries.

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[2] Osborn, H. T., *Phytopath.*, 1935, **25**, 160.

[3] Wyckoff, R. W. G., and Lagsdin, J. B., *Rev. Sci. Instr.*, 1937, **8**, 74, 127.

Similar ultracentrifugal procedures have not isolated homogeneous macromolecules from the juice of healthy tobacco plants. If such proteins exist, they are either highly unstable or are present only in minute amounts.

9806

Toxicity of Nicotinic Acid.

K. K. Chen, Charles L. Rose and E. Brown Robbins.
From Lilly Research Laboratories, Indianapolis.

Nicotinic acid (I), chemically pyridine-β-carboxylic acid, was prepared as early as 1867 by Huber[1] from the oxidation of nicotine (II). During the early investigation of vitamin B, nicotinic acid was isolated from rice polishings,[2, 3, 4] and suspected to have an antineuritic action in pigeons, although subsequent tests definitely excluded this possibility.[5] A renewal of interest was aroused by the publication of Elvehjem, Madden, Strong, and Woolley,[6] who showed that nicotinic acid or its amide isolated from the liver extract cures canine blacktongue of the Goldberger type.[7] Their

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[1] Huber, C., *Liebigs Ann. Chem.*, 1867, **141**, 271; *Ber. deut. chem. Gesellsch.*, 1870, **3**, 849.

[2] Funk, C., *J. Physiol.*, 1911-12, **43**, 395; 1912-13, **45**, 489; 1913, **46**, 173.

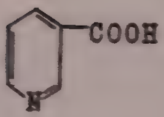
[3] Suzuki, U., Shimamura, T., and Odake, S., *Biochem. Z.*, 1912, **43**, 89.

[4] Drummond, J. C., and Funk, C., *Biochem. J.*, 1914, **8**, 598.

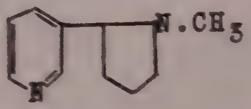
[5] Funk, C., *J. A. M. A.*, 1937, **109**, 2086.

[6] Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W., *J. Am. Chem. Soc.*, 1937, **59**, 1767; *J. B. C.*, 1938, **123**, 137.

[7] Smith, D. T., *J. A. M. A.*, 1937, **109**, 2086.



(I)



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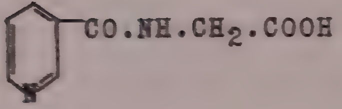

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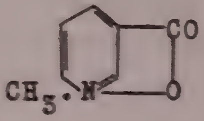

(IV)

work has been confirmed by Street and Cowgill.[8] Favorable clinical results in the treatment of human pellagra with nicotinic acid have already been obtained by Spies, Cooper, and Blankenhorn,[9] Fouts, Helmer, Lepkovsky, and Jukes,[10] Smith, Ruffin, and Smith,[11] and Margolis, Margolis, and Smith.[12]

Pharmacologically, Hunt and Renshaw[13] failed to observe any effect upon the autonomic nervous system with nicotinic acid and its derivatives. In dogs Ackermann[14] and Komori and Sendju[15] reported that nicotinic acid is excreted in urine partly as nicotinyl glycine (III) and trigonelline (IV), and partly unchanged. It is also apparent from these publications that nicotinic acid has a relatively low toxicity.

In view of the present interest in nicotinic acid (I) for the management of pellagra, and its suggestive relationship, although chemically remote, to nicotine (II), it appears desirable to have some precise information concerning its toxicity in animals. The sample of nicotinic acid we investigated was entirely colorless and melted at 236-237° C. (corrected). The solutions employed for injection were neutralized with adequate amounts of sodium carbonate—a

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[8] Street, H. R., and Cowgill, G. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 547.

[9] Spies, T. D., Cooper, C., and Blankenhorn, M. A., *J. Am. Med. Assn.*, 1938, **110**, 622 and 766.

[10] Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 405.

[11] Smith, D. T., Ruffin, J. M., and Smith, S. G., *J. A. M. A.*, 1937, **109**, 2054.

[12] Margolis, L. H., Margolis, G., and Smith, S. G., cited by Smith *et al.*

[13] Hunt, R., and Renshaw, R. R., *J. Pharmacol. and Exp. Therap.*, 1929, **35**, 75; 1929, **37**, 177.

[14] Ackermann, D., *Z. f. Biol.*, 1912, **59**, 17.

[15] Komori, Y., and Sendju, Y., *J. Biochem.* (Japan), 1926, **6**, 163.

1% solution having a pH of 7.4, one of 10% that of 7.8, and one of 20% that of 8.1. Minimal lethal doses were determined in white mice, white rats, and guinea pigs by intravenous injection with nicotine and nicotinic acid, respectively. The body weight of mice varied from 15 to 20 gm., that of rats 80 to 110 gm., and that of guinea pigs from 250 to 280 gm. As shown in Table I, nicotine is approximately 5625 times as toxic as nicotinic acid in mice, 3500 times as toxic in rats, and 778 times as toxic in guinea pigs. With lethal or near-lethal doses of both nicotine and nicotinic acid, all animals developed clonic convulsions, and either died promptly or recovered without apparent after effects.

TABLE I.
Acute Toxicity of Nicotine and Nicotinic Acid.

Compound	Species	Dose, mg. per kg.	No. of Animals used	No. died	Minimal lethal dose, mg. per kg.
Nicotine	**Mice**	0.4	1	0	0.8
		0.5	1	0	
		0.6	6	0	
		0.7	7	0	
		0.8	10	7	
		0.9	5	5	
	Rats	0.5	1	0	1.0
		0.7	5	0	
		0.8	5	2	
		0.9	5	2	
		1.0	5	3	
		1.1	5	5	
		1.2	3	3	
	Guinea Pigs	3.0	4	1	4.5
		4.0	3	0	
		4.5	4	3	
		5.0	3	3	
Nicotinic Acid	**Mice**	2500.0	3	0	4500.0
		3000.0	3	0	
		4000.0	3	0	
		4500.0	3	3	
	Rats	3000.0	5	2	3500.0
		3500.0	5	3	
	Guinea Pigs	3000.0	3	0	3500.0
		3500.0	5	3	

A group of 5 mice, injected daily by the tail vein, each with 500 mg. of nicotinic acid per kg. of body weight for 4 weeks except Saturdays and Sundays, survived with an increase in body weight.

Two adult dogs, weighing 13.8 and 15 kg., respectively, each received daily *per os* 2 gm. of nicotinic acid dispensed in capsules. The smaller animal began to excrete bloody feces on the 11th day, developed a series of convulsions on the 12th day, became increasingly irritable and bit the attendants. Since it was suspected of having rabies, medication was stopped on the 13th day. The dog was found dead on the 19th day, with a total loss of 4.2 kg. of body weight, not from rabies but apparently from poisoning due to nicotinic acid. The larger dog received a total amount of 40 gm. of nicotinic acid in the course of 20 days, gradually lost appetite until it refused to eat, and began to have occasional convulsions after the second week of experimentation. On the 20th day, the convulsions, tonic in character, became increasingly frequent, each series lasting from 1 to 4 minutes. The animal was unable to stand up during interconvulsive periods. Bloody feces were expelled. The condition was so critical that it was considered advisable to sacrifice the animal in order to avoid postmortem changes. The total loss of body weight in this animal was 3.6 kg. Autopsies were performed by Dr. Paul N. Harris. In the smaller dog, there were fatty metamorphosis of the liver and intussusception of small intestines which apparently existed for some time but was not sufficient to account for the death. In the larger dog, there were 6 erosions, 2 to 5 mm. in diameter, in the lesser curvature of the stomach which was filled with some bloody fluid; slight hyperemia of both small intestines and colon; petechiæ in the mucosa of the colon; a small amount of blood in colon contents; and fatty metamorphosis of the liver. Perhaps a more significant finding was the fact that in both dogs a few ganglion cells of the cortex, hippocampus and basal ganglia were shrunk and deeply stained. If this indicated an injury caused by excessive doses of nicotinic acid, it might be then that the convulsions observed before death were merely its manifestations. Further investigation will be necessary before this point can be settled.

Four additional dogs were given daily for a period of 8 weeks, except Saturdays and Sundays, smaller doses by mouth. They were 1, 0.5, 0.2, and 0.06 gm., respectively. At the end of the 8th week, all 4 animals appeared in good health and had gained in weight. A trace of albumin was found twice in the urine of the dog on the 1 gm. dose and in that of the dog on the 0.5 gm. dose. A trace of sugar was also detected once in the urine of both animals.

Unlike nicotine, nicotinic acid has no action upon the autonomic ganglia as already proved by Hunt and Renshaw.[14] A concentration of 1:5,000 of the acid did not elicit any response from the isolated rabbit's intestine. Similarly, a solution of 1:2,000 was inert to the isolated guinea pig's uterus. An intracutaneous injection of 0.1 cc.

of a 1% solution of nicotinic acid was followed by no hyperemia or ulceration. The substance has therefore little irritating action. Since nicotinic acid is often prepared from nicotine,[16] caution should certainly be exercised to eliminate any possibility of contamination by the alkaloid. Simple animal tests will detect such an impurity.

Summary. Nicotinic acid is at least several hundred times less toxic in mice, rats, and guinea pigs than nicotine. Nicotinic acid is devoid of action upon the autonomic ganglia. Nevertheless, repeated administration of large doses, 2 gm. daily, in dogs has resulted in poisoning and deaths.

9807

Renal Excretion of Exogenous Creatinine in the Aglomerular Toadfish, *Opsanus tau.*

JAMES A. SHANNON.

From the Marine Biological Laboratories, Woods Hole, and the Department of Physiology, New York University College of Medicine.

We have presented elsewhere a quantitative analysis of the relationship between plasma concentration and urine flow, and the tubular excretion of phenol red by the aglomerular fishes, *Lophius piscatorius* and *Opsanus tau.*[1] It was shown, in each of these species, that above 6.0 mg. % plasma phenol red there was no significant increase in the rate of excretion and that this maximal rate was not influenced by the extent or direction of the diffusion gradient. The present study represents an attempt at a similar analysis of the tubular excretion of exogenous creatinine by *Opsanus.* Marshall and Grafflin[2] have shown in this species that the fraction of injected creatinine which is excreted in an 18-hour period decreases progressively with increasing amounts of injected creatinine; furthermore, the fraction of a small injection excreted in a given period of time increases with increasing urine flow.

The present experiments may be divided into 2 groups. In the first group one urine collection period, 10 to 12 hours in duration and beginning 8 to 12 hours after the injection of creatinine, was made after single doses of 50 to 1500 mg. per kg. Under these conditions the rate at which the plasma concentration falls is so slow that a single blood sample drawn at the end of the urine collection period suffices for the calculation of the clearance. Observations of this nature on 81 fish are given in Fig. 1 and summarized in Table I. In the second group of experiments, an effort was made to obtain in the same fish 2 urine collection periods at high plasma creatinine levels but at different rates of urine flow. An initial period was taken as described above, after which a second injection of creatinine (400 to 1000 mg. per kg.) was usually given and the second urine collection period started about 10 hours later. Blood was drawn at the end of each period. The initial periods of these experiments, 16 in number, are included in Fig. 1 and Table I. Blood and urine collections were made as previously described.[1] Creatinine was determined on Folin Wu filtrates of plasma and diluted urine by the Folin method.[3]

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[16] McElvain, S. M., *Organic Synthesis,* 1925, 4, 49.

[1] Shannon, J. A., *J. Cell. and Comp. Physiol.,* 1938, in press.

[2] Marshall, E. K., Jr., and Grafflin, A. L., *J. Cell. and Comp. Physiol.,* 1932, 1, 161.

In confirmation of Marshall and Grafflin, we find that the rate of excretion of creatinine does not increase in direct proportion to the plasma concentration, and that the rate of excretion is related to urine flow. The data presented in Fig. 1 and summarized in Table I suggest the presence of an excretion maximum, but this is not as

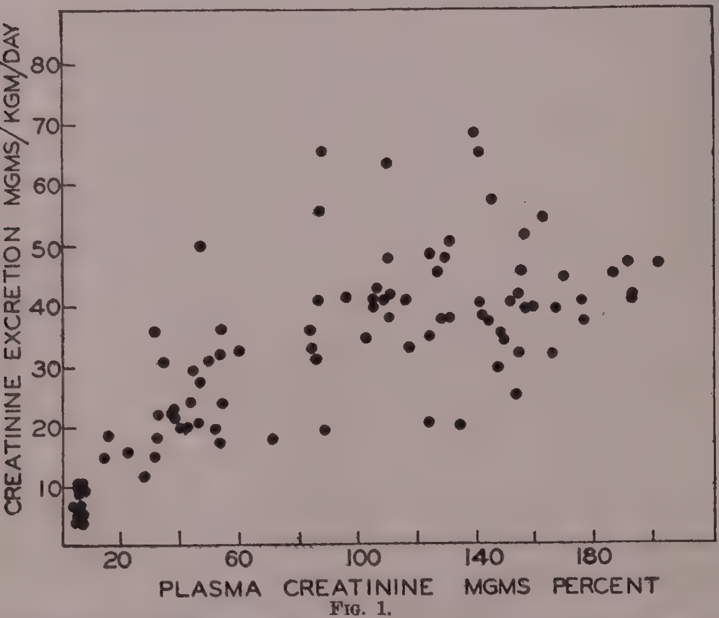

FIG. 1.

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[3] Folin, O., *J. Biol. Chem.*, 1919, **38**, 81.

TABLE I.

Summary of the data shown in Fig. 1.

No. of periods	Mean plasma concentration. mg. %	Mean rate of excretion. mg. per kg. per day
9	4.81	6.28
11	16.6	17.02
11	38.5	23.9
11	56.0	28.4
11	93.4	41.3
11	117.2	41.9
11	136.5	45.0
11	154.0	41.5
11	181.0	43.0

sharply defined as in the case of phenol red. The scatter of the data is not due primarily to differences in urine flow since this does not vary widely; rather it appears that there is considerable variation in the capacity of the kidneys of similarly sized fish to secrete creatinine. The variation observed here is no greater than that previously reported for the tubular excretion of phenol red in this species and in *Lophius*, where urine flow has no demonstrable effect upon the rate of tubular excretion.

There is no doubt that an increase in urine flow is accompanied by an increase in creatinine excretion. This fact is evident not only in individual experiments but also in the summary in Table II. In view of this relationship it was not possible to utilize two-period experiments for the study of the excretion maximum, since the second period invariably had a higher urine flow. But the two-period experiments do show that the increase in creatinine excretion, with increased urine flow, is greatest when an increase in plasma concentration accompanies the increase in urine flow, and is greater than would be expected from either the increase in urine flow (Group 2, Table II) and the increase in plasma concentration (Fig. 1, Table I).

It is not known why urine flow influences the rate of tubular ex-

TABLE II.

Summary of Data Showing Effect of Urine Flow on Rate of Creatinine Excretion.

No. of Experiments in each group	Urine Flow cc. per kg. per day		Plasma Creatinine mg. %		Creatinine Excretion mg. per kg. per day		Ratio Second Period / First Period
	First Period	Second Period	First Period	Second Period	First Period	Second Period	
4	5.79	25.3	147.7	87.8	45.1	55.3	1.23
7	8.63	18.6	145.3	164.3	43.4	69.7	1.61
5	5.16	14.8	103.0	235.0	46.0	118.8	2.58

cretion of creatinine, but it may be noted that the aglomerular tubule must excrete its own water, this water moving in the same direction as the excreted solutes. Whether this circumstance imposes a relationship upon the mechanism of transfer which is absent in the glomerular nephron cannot be determined at present. If the transport of creatinine and water are independent functions, it is possible that the concentration of creatinine in the final tubular urine imposes a limitation upon its excretion. There would appear to be no reason to postulate a different mechanism of excretion in the aglomerular and glomerular nephron; but if such a relationship exists in the latter it may not be discoverable since the creatinine is excreted into a relatively large quantity of glomerular filtrate which subsequently undergoes concentration by the tubular reabsorption of water.

9808 P

Action of High Pressures on Plant Viruses.

J. Basset, A. Gratia, M. Macheboeuf and P. Manil. (Introduced by J. H. Northrop.)

From the Institut de Bacteriologie de l'Universite de Liege, Institut Pasteur de Paris, Institut Agronomique de l'Etat à Gembloux.

Two of us (J. Basset and M. Macheboeuf) with the help of several coworkers[1] have submitted different biological samples to the action of an apparatus devised several years ago by Basset[2] which realizes enormous pressures (beyond 20,000 atmospheres). Non-sporulated bacteria were killed after 45 minutes of exposure to a pressure around 5,000 atmospheres, while sporulated bacteria resist as high pressures as 20,000 atmospheres. Certain viruses and bacteriophages, on the contrary, are inactivated at pressures around 3,000-4,000 atmospheres, while some enzymes are inactivated at about 13,000 atmospheres and toxins at 17,000-19,000 atmospheres. Thus, if toxins behave much like enzymes, bacteriophages behave more like viruses. Globulins are denatured around

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[1] Basset, J., Macheboeuf, M., and coworkers, *Compt. rend.*, 1932, **195**, 1431; 1933, **196**, 67, 1138, 1540; **197**, 796; 1935, **200**, 496, 1072, 1247, 1882; 1936, **202**, 121; *Bull. soc. philomath.*, 1933, 575; *Ergebnisse der Enzymforschung* (Leipzig), 1931, **3**, 304; *Bull. soc. chim. biol.*, 1936, **18**, 1181.

[2] Basset, J., *Compt. rend.*, 1927, **185**, 343; 1930, **191**, 928.

8,000 atmospheres while albumins usually resist much higher pressures.

On the other hand, two of us (Gratia and Manil)[3] studying the antigenic properties of plant viruses have found that there exist a few which are not only neutralized by the action of corresponding anti-serums but are also heavily and specifically flocculated, while most of them are neutralized without exhibiting any precipitation. In the first group are the common mosaic of tobacco and the X virus of potato, while in the second are the virus of tobacco necrosis and Y virus of potato. The heavy flocculation of the first ones is caused by the presence of a heavy active protein which was purified into a crystalline form by chemical means,[4, 5] and by ultra-centrifugation.[6, 7]

It seemed interesting to test the resistance to high pressures of both types of plant viruses, either pure or mixed. The results of 2 experiments follow:

Active, specific protein of tobacco mosaic virus, obtained by differential ultra-centrifugation, was divided into 5 parts. One remained untreated and served as a control; the 4 others were pressed for 45 minutes respectively at 2,000, 4,000, 6,000 and 8,000 atmospheres. The control and each of the 4 pressed samples were then divided in 3 portions. To the first was added an equal volume of anti-tobacco mosaic serum for the flocculation test; to the second was added a quarter volume of saturated ammonium sulfate solution containing 5% acetic acid for the crystallization test, and the third portion was inoculated on leaves of *Nicotiana glutinosa* for the virulence test. The results were as follows: Flocculation test was much alike for the control and for the samples pressed at 2,000, 4,000 and 6,000 atmospheres, *i. e.*, there appeared a heavy precipitate in the form of a gel much like a clot that slowly retracts; the mass of the gel was somewhat smaller for the samples treated at 4,000 and 6,000 atmospheres. On the contrary, the sample pressed at 8,000 atmospheres presented a very striking difference, the precipitate being reduced to a few small clumps which sedimented readily. Similarly, the crystallization test was much alike for the control and the 3 first pressed samples while the last one, pressed at 8,000 atmospheres, exhibited an amorphous precipitate in which only a very few crystalline forms could still be seen. The virulence test gave the following number of lesions: 90, 49, 63, 58, and 2 respectively for the control and the 4 pressed portions.

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[3] Gratia, A., and Manil, P., *Compt. rend. soc. biol.*, 1933, **114**, 923, 929, 1382; **115**, 189, 1239; 1934, **117**, 490, 493; **118**, 379; 1936, **122**, 814; **123**, 325, 509; 1937, **126**, 67; *Bull. l'acad. roy. med. Belg.*, 1935, 208.

[4] Stanley, W. M., *Science*, 1935, **81**, 644; 1936, **83**, 85, 626; *Phytopathology*, 1935, **25**, 922; 1936, **26**, 305.

[5] Bawden, F. C., Pirie, N. W., Bernal, J. D., and Fankuchen, I., *Nature*, 1936, **138**, 1051; Bawden, F. C., and Pirie, N. W., *Proc. Roy. Soc.* (London), 1937, **B 123**, 272.

[6] Wyckoff, R. W. G., Biscoe, J., and Stanley, W. M., *J. Biol. Chem.*, 1937, **117**, 57.

[7] Gratia, A., and Manil P., *Compt. rend. soc. biol.*, 1937, **127**, 423.

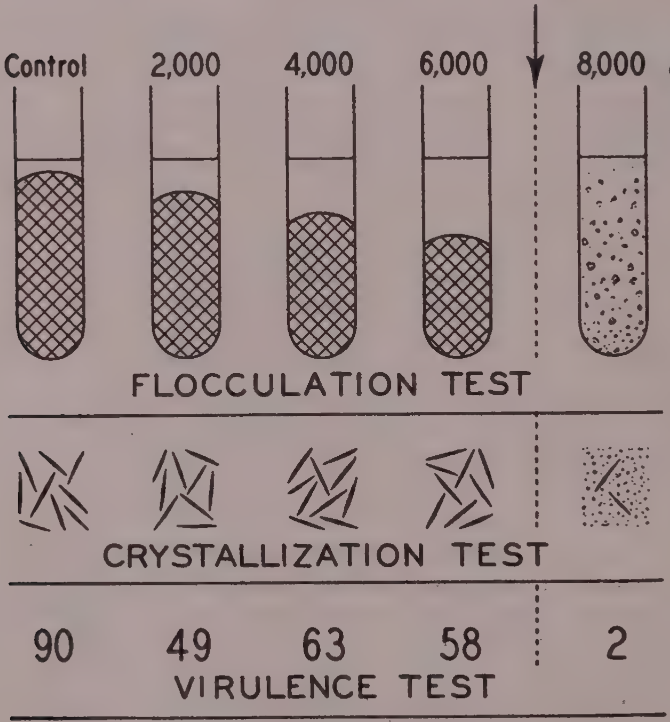

FIG. 1.

It is evident that the specific protein resists apparently up to a pressure of 6,000 atmospheres but undergoes a marked change at 8,000 atmospheres. This change parallels the serological, chemical, and virulence test.

The next experiment was then performed comparatively with plain unpurified juices of tobacco plants infected either with tobacco mosaic virus or with tobacco necrosis virus, a virus which is neutralized by the corresponding antiserum but which does not show any precipitation nor has it been obtained as a crystalline protein.

The virulence test on *Nicotiana tabacum* was performed for tobacco necrosis and on *Nicotiana glutinosa* for tobacco mosaic and gave the results shown in Table I.

TABLE I.

	Control	3,000 atmospheres	5,500 atmospheres	8,000 atmospheres
Tobacco necrosis on *Nicotiana tabacum*	lesions	lesions	0	0
Tobacco mosaic on *Nicotiana glutinosa*	66	121	90	39

The second experiment confirmed the first one in regard to tobacco mosaic virus, the destructive action of pressure being more than 8,000 atmospheres which is well above the usual lethal pressure for certain viruses and close to the denaturing pressure for globulin. On the other hand, tobacco necrosis is inactivated between 3,000 and 5,000 atmospheres, the usual virus lethal pressure.

It might be of interest to note that in both experiments the number of lesions for tobacco mosaic was somewhat higher after the virus had been pressed between 3,000 and 6,000 atmospheres than when pressed at lower or higher pressures. This fact might be of importance if duplicated.

9809

Effect of Autoclaving on Vitamin Potency of Nicotinic Acid.*

SUSAN GOWER SMITH, GEORGE MARGOLIS AND LESTER H. MARGOLIS. (Introduced by D. H. Sprunt.)

From the Department of Medicine, Duke University School of Medicine, Durham, N. C.

Smith and Hendrick[1] and Goldberger, Wheeler, Lillie, and Rogers[2] of the U. S. Public Health Service showed that the vitamin B value of yeast, as then understood, was due, in part, to the antineuritic substance which was destroyed by autoclaving, and, in part,

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* Part of the expenses of this work was covered by grants from the Rockefeller Foundation and the Lederle Biological Laboratories.

[1] Smith, M. I., and Hendrick, *U. S. P. H. Rep.*, 1926, **41**, 201.

[2] Goldberger, Jos., Wheeler, G. A., Lillie, R. D., and Rogers, L. M., *U. S. P. H. Rep.*, 1926, **41**, 297.

to a more heat-stable factor which was able to withstand autoclaving. The term "autoclaving" is used advisedly, since evidence submitted by Kennan, Kline and Elvehjem[3] indicates that the antineuritic factor is more stable to dry heat than is the factor which withstands autoclaving. Goldberger and his associates demonstrated to the satisfaction of most investigators that pellagra is a vitamin deficiency disease, apparently due to a lack of the factor in yeast which is uninjured by autoclaving. Since canine blacktongue develops on the same type of deficiency which produces pellagra, is characterized by similar symptoms and is cured by the same food factors, it is reasonable to conclude that it is an analog of pellagra. Consequently many tests for curative factors have been carried out on dogs suffering from artificially produced blacktongue.

The observation by Elvehjem, Madden, Strong, and Woolley[4] that nicotinic acid (the simple pyridine carboxylic acid) would cure blacktongue in dogs has been corroborated by Dann,[5] Street and Cowgill.[6] It was soon demonstrated that it had a similar curative value in human pellagra by Fouts, Helmer, Lepkovsky and Jukes,[7] Smith, Ruffin and Smith,[8] and Spies.[9]

The question naturally arises as to whether or not the curative properties of nicotinic acid are decreased or destroyed by autoclaving. The following experiments were designed to answer this question:

Ten dogs were allowed to develop blacktongue on a modification of Goldberger's diet No. 123, as used in this laboratory, the details of which were published recently by Smith, Persons and Harvey.[10] When blacktongue was well developed the animals were divided into 5 groups of 2 dogs each, paired according to weight. Dog No. 1 in each group was given autoclaved nicotinic acid and dog No. 2 the unautoclaved. The concentration found most satisfactory was a solution of 5 mg. per ml. For oral use the acid was dissolved in distilled water and for parenteral use in sterile saline. The nicotinic acid solution was autoclaved for 2½ hours at 15 lb. pressure and a second portion for 5 hours at 15 lb. pressure. Only 2 dogs were given the material autoclaved for 2½ hours; since the 5-hour material proved equally effective it seemed unnecessary to test further the nicotinic acid which had been autoclaved for only 2½ hours. The dose varied from 1 to 10 mg. per kilogram given daily for a 10-day period. Previous tests had indicated that within these limits the size of the dose makes little if any difference.

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[3] Kennan, J. A., Kline, O. L., and Elvehjem, C. A., *J. Nutrition*, 1935, **9**, 63.

[4] Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W., *J. Am. Chem. Soc.*, 1937, **59**, 1767.

[5] Dann, W. J., *Science*, 1937, **86**, 616.

[6] Street, H. R., and Cowgill, G. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 547.

[7] Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, J. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 405.

[8] Smith, D. T., Ruffin, J. M., and Smith, S. G., *J. Am. Med. Assn.*, 1937, **109**, 2054.

[9] Spies, T. D., *Lancet*, 1938, **234**, 252.

[10] Smith, D. T., Persons, E. L., and Harvey, H. I., *J. Nutrition*, 1937, **14**, 373.

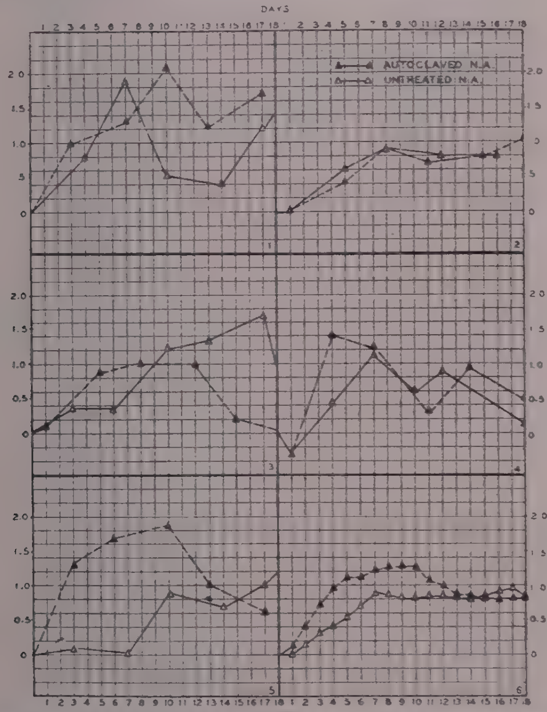

CHART I

Weight Curves of Dogs with Experimental Canine Blacktongue Treated with Nicotinic Acid for Ten Days.

Fig. 1. Dogs receiving 1.0 mg. N.A. per kilo of body weight.
Fig. 2. ” ” 2.0 ” ” ” ” ” ” ”
Fig. 3. ” ” 10.0 ” ” ” ” ” ” ”
Fig. 4. ” ” 1.5 ” ” ” ” ” ” ”
Fig. 5. ” ” 1.5 ” ” ” ” ” ” ”
Fig. 6. Each curve represents an average of the five dogs in the preceding figures.

Results. Mouth symptoms healed quickly with both the autoclaved and unautoclaved nicotinic acid. There was an almost immediate increase in weight as shown in Chart 1. No difference could be detected between the curative action of the autoclaved and that of the unautoclaved solution. The average length of time at which blacktongue symptoms reappeared after treatment ended was 17 days for the autoclaved and 16 days for the unautoclaved solution.

We conclude, therefore, that nicotinic acid has little if any of its vitamin potency destroyed by autoclaving. It thus resembles in this respect, as well as others, the P.P. factor of Goldberger.

9810 P

Successful Transmission of Cutaneous Leishmaniasis by the Bites of *Stomoxys calcitrans*.

D. A. BERBERIAN. (Introduced by E. W. Dennis.)

From the Department of Parasitology, the American University of Beirut, Beirut, Lebanon.

The experiments carried out by Sergent, Sergent, Parrot, *et al.*,[1] Adler and Theodor,[2, 3, 4] incriminated the sandfly as the probable vector of cutaneous leishmaniasis. This hypothesis has been supported by the fact that it has been possible to infect sandflies under experimental conditions, and to produce the cutaneous lesions by the injection of crushed infected sandflies. Furthermore, the sandfly theory of transmission of cutaneous leishmaniasis has been attractive because of the readiness with which leishmanias exflagellate and multiply in the alimentary tract of these insects, and the epidemiological evidence apparent in the close correspondence of the distribution of certain species of sandflies with the distribution of cutaneous leishmaniasis. However, we have not been entirely satisfied with the sandfly theory of transmission because of the following facts:

1. Critical experiments designed to demonstrate the transmission by the bite of the sandfly have regularly failed. Although it is conceivable that infection may be accomplished by crushing an infected sandfly at the site of the bite, the likelihood of this occurring would appear to be rather remote to any person who has attempted to destroy a sandfly while in the act of biting.

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[1] Sergent, Ed. et Et., Parrot, L., Donatien, H., et Beguet, M., *Arch. Sc.*, 1921, **173**, 1030.

[2] Adler, S., and Theodor, O., *Ann. Trop. Med. and Parasit.*, 1925, **19**, 365.

[3] *Ibid.*, 1926, **20**, 175.

[4] *Ibid.*, 1927, **21**, 89.

2. There is no evidence that sandflies are attracted to lesions as preferential sites for feeding, and, as *L. tropica* is confined to the lesions, the probability of a sandfly acquiring the infection would be remote even though there was ample opportunity to feed on persons exhibiting suitable lesions.

3. Should a sandfly actually feed from the oriental sore under natural conditions, it should be remembered that the parasites are limited to the endothelial cells, and the likelihood of infected tissue cells being ingested with the blood must be rather small.

4. Data published by Smith, Mukerjee, *et al.*,[5] indicate that only 25% of sandflies infected on their first bite, and few, if any, infected on their second or third feeding would survive for the period of 6-8 days required for *L. tropica* to become infective in *Phlebotomus sergenti*[6] and *P. papatasii*[4] respectively.

Attracted by the possibility of sucking or biting flies, which are commonly attracted to skin lesions, transmitting oriental sore and kala azar mechanically, we have carried out experiments using *S. calcitrans,* house flies and bed bugs. We have successfully transmitted cutaneous leishmaniasis by the bites of *S. calcitrans.*

The source of infection consisted of a lesion produced on the author by the intracutaneous inoculation of 0.1 cc. of 14-day-old culture of *L. tropica.* The lesion first appeared about 15 days after inoculation, and was used during the period 80-90 days after inoculation.

On July 11, 1937, two stable flies were fed on the lesion and were immediately transferred to the anterior aspect of the left thigh of volunteer No. 2. Each fly bit once after being transferred. On July 20, 1937, one fly was fed on the lesion and immediately placed on volunteer No. 2 at approximately the same place used for the feeding on July 11th; the fly bit twice. On July 21st, four flies were fed on the lesion and immediately transferred to the volunteer as on 2 previous occasions; the 4 flies bit a total of 7 times. The volunteer was bitten a total of 11 times by 7 flies.

A pin-point violaceous papule appeared at the site of the bites on the volunteer 5 months after the last feed; the lesion increased in size, and on January 20, 1938, was 2 mm. in diameter. On January 22nd, six months after feeding, a second papule appeared near the first lesion. Examination of a smear prepared from the first lesion on January 24th showed numerous Leishman-Donovan bodies.

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[5] Smith, R., Mukerjee, S., Halder, K. C., and Chiranji Lal, J., *Ind. J. Med. Res.*, 1936, **24**, 295.

[6] Adler, S., and Theodor, O., *Ann. Trop. Med. and Parasit.*, 1929, **23**, 1.

Thus at least 2 positive transmissions have resulted from 11 bites by only 7 flies, and it is possible that lesions will develop at the site of other bites.

We may conclude that transmission of oriental sore by means of stable flies is possible, and in view of the ease of transmission, it seems probable that transmission by this method frequently occurs under natural conditions. A complete report on our experiments using *S. calcitrans* and other insects will be published later. We believe that *S. calcitrans* and similar blood-sucking flies may be capable of transmitting visceral leishmaniasis under natural conditions, and experiments to test this hypothesis are included in our program.

9811 P

The Diagnosis of Venous-arterial Shunt by Ether Circulation Time Method.

WILLIAM BENENSON AND WILLIAM M. HITZIG. (Introduced by George Baehr.)

From the Medical Service, Queens General Hospital, and the Medical Service and Cardiographic Laboratory of the Mount Sinai Hospital, New York City.

Recently McGuire and Goldman[1] showed that in certain types of congenital heart disease the arm to carotid sinus circulation time was considerably abbreviated. They regarded this apparent increase in the velocity of blood flow as characteristic of venous-arterial shunt. To their interesting observations we wish to add our findings with the ether[2] and saccharin[3] circulation time methods, which we believe offer conclusive evidence for the presence of right to left shunt.

Ether (5 minims) injected into an antecubital vein in normal individuals enters the right heart whence it reaches the pulmonary capillary bed and becomes volatilized. The time elapsing between the injection and the detection of the ether smell in the nose represents the arm to lung time, which in normal individuals and in those with circulatory failure is always a fraction of the arm to tongue time as measured by the saccharin method. However, when a similar or lesser quantity of ether and the usual amount of saccharin were injected separately into cyanotic patients suffering from congenital heart disease, the following circulatory phenomena became manifest in 4 subjects in whom venous-arterial shunt existed (Table I).

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[1] McGuire, J., and Goldman, F., *Am. Heart J.*, 1937, **14**, 230.

[2] Hitzig, W. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 935; *Am. Heart J.*, 1935, **10**, 1080.

[3] Fishberg, A. M., Hitzig, W. M., and King, F. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 651.

TABLE I.
4 Cases with Tetralogy of Fallot.

Case No.	Date	Minims ether injected	Age	Sex	Arm to Lung Time in Seconds (Ether)	Arm to Tongue Time in Seconds (Saccharin)	Paraesthesias
1.	4/34	5	11	F.	7.5	3-4	Generalized Tingling ++++
2.	6/37	5	30	F.	11.0	11.8	Generalized Tingling ++++
3.	1/38	1	3½	M.	10	*	Marked Paraesthesia of face +++
4.†	2/38	3	9	M.	20	*	Marked Paraesthesia of scalp ++

*Because of the age and lack of cooperation of the patients the saccharin test was not performed.

†The authors are indebted to Dr. Betty Huse for her assistance in observing this case.

(1) Alteration of the usual relationship between the arm to lung and the arm to tongue circulation times from a fractional relationship to a ratio of 1 to 1, or 1+ to 1. (Cases 1 and 2.)

(2) Shortening of the arm to tongue time similar to the shortened arm to carotid sinus time of McGuire and Goldman[1] as in Case 1. In Case 2 the clinical picture was that of heart failure erroneously attributed to rheumatic heart disease and the relatively rapid saccharin time suggested venous-arterial shunt in congenital heart disease.

(3) Stinging and tingling sensations of a few seconds' duration which are either localized to the face and scalp or generalized extending in wave-like fashion to the more distant portions of the body. The severity of this cutaneous reaction was proportional to the quantity of ether injected, the most dramatic reaction being obtained when the full dose of 5 minims of ether was employed. These paræsthesias appeared simultaneously with the detection of ether

by smell in 3 instances, although in case (1) they definitely antedated the ether end-point and corresponded more closely to the shortened arm to tongue time.

Ordinarily when ether is injected it volatilizes and escapes almost completely from the lung during the first circuit of the blood. Only a negligible amount may remain and eventually reach the systemic capillaries. However, in the presence of a venous-arterial communication, ether in variable amount depending upon the anatomic defect, is shunted into the left side of the heart from where it is propelled into the systemic circuit setting up the above described irritative phenomena in the capillaries of the skin. The rapid saccharin time is similarly explained.

Circulatory studies with saccharin and ether have been performed by us in over 2,000 patients including 2 cases with acquired septal defect due to coronary thrombosis and other congenital cardiacs with and without cyanosis. In no other case have we encountered the above sensory and circulatory disturbances. The ether method is of definite value, therefore, in establishing with certainty the existence of venous-arterial shunt.

9812 P

Xylose, Inulin, and Creatinine Clearance in the Normal Frog.

ROY P. FORSTER. (Introduced by M. F. Guyer.)

From the Laboratories of Zoology and Physiological Chemistry, University of Wisconsin.

Hendrix, Westfall, and Richards[1] have shown from microanalyses of glomerular urine obtained directly from the exposed kidney of the frog, after intravenous injection of inulin, that it contained the same concentration of inulin as the plasma. Also, Richards and his coworkers[2] demonstrated in a double perfusion experiment that the frog's kidney was unable to excrete inulin when the fluid flowing through the intertubular capillaries contained inulin and that of the glomerular capillaries did not. Creatinine was found by them to undergo insufficient concentration in the frog renal tubules to suggest secretion.

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[1] Hendrix, J. P., Westfall, B. B., and Richards, A. N., *J. Biol. Chem.*, 1936, **116**, 735.

[2] Richards, A. N., and Walker, A. M., *Am. J. Med. Sci.*, 1935, **190**, 727.

The following experiments on normal unanesthetized bullfrogs (*Rana catesbiana*) were designed to determine the relation of xylose to inulin clearance, the relationship between plasma concentration of inulin and the amount excreted per unit time, and the relationship between creatinine and inulin clearances. A total of 16 normal bullfrogs, varying in weight from 280 to 600 gm. were used. Between and during collections they were kept individually in large battery jars almost completely immersed in tap water. Exogenous creatinine, inulin, xylose, and water were injected into the dorsal lymph sac. Urine was collected by catheter at half-hour or hour intervals and blood was withdrawn directly from the heart before and after each experiment.

In 9 individuals xylose was found to have a lower clearance than inulin, the average xylose to inulin ratio being 0.80. In 27 individual collection periods on 7 animals with the plasma level of exogenous creatinine varying from 11.7 to 260 mg. %, and with the urine flow ranging from 3.1 to 27.8 cc. per kg. per hour the creatinine clearance remained practically identical with that of

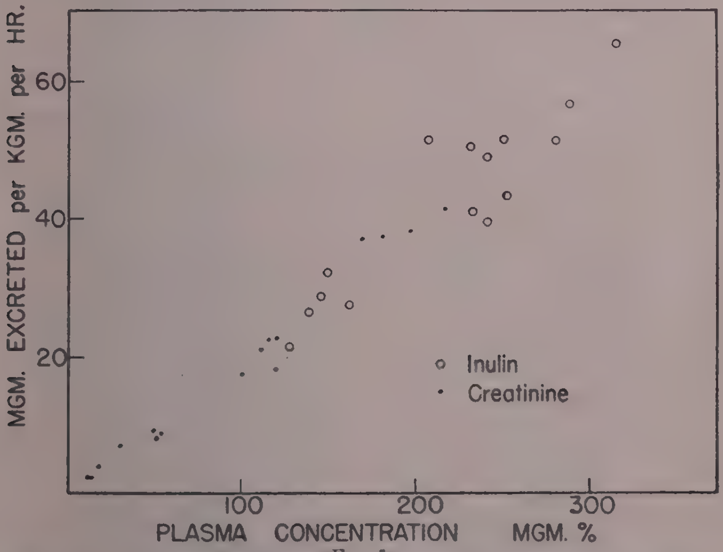

FIG. 1.

Data on 6 normal unanesthetized animals showing that the rate of inulin and creatinine excretion is proportional to the plasma level. As renal clearance in frogs varies with the urine flow these data represent all collections within the limited range of 10 to 18 cc./kg./hr. The absence of a curvilinear relationship between plasma level and amount excreted per unit time indicates that tubular secretion is not a factor in the renal elimination of these two substances. Scattering of the data is due to variations in urine flow, which in these animals is almost entirely a glomerular phenomenon.

inulin. The average creatinine/inulin clearance ratio of all collections was 0.996, with a standard deviation of 0.0643.

The clearance of both inulin and creatinine is not constant for any one animal, but varies with the urine flow. The average clearance at a urine flow of 1 cc. per kg. per hour is 3.5 cc. per kg. per hour, and this rises to an average of 20 at a urine flow of 15. When collection periods are selected within a limited range of urine flow, and plasma concentration of inulin and creatinine then plotted against the amount excreted per unit time the data describes a straight line, extrapolating to the zero axis, as illustrated in Fig. 1. This direct proportion to plasma level of inulin and creatinine, and the identical clearances of these substances under all circumstances investigated, is interpreted as indicating that they are excreted solely by glomerular filtration, and that tubular secretion is not a factor in their elimination.

9813 P

Partial Reactivation of Formolized Tobacco Mosaic Virus Protein.*

A. FRANK ROSS AND W. M. STANLEY. (Introduced by Richard E. Shope.)

From the Rockefeller Institute for Medical Research, Princeton, N. J.

A marked reactivation of tobacco mosaic virus protein that has been partially or completely inactivated by formaldehyde[1] has been obtained by dialysis at pH 3. In the experiments to be reported, inactivation was allowed to proceed at room temperature in reaction mixtures containing 2% purified virus protein and 2% formaldehyde in M/10 phosphate at pH 7. Samples were removed after different periods of time and immediately dialyzed against cold distilled water for about 6 hours in order to stop the reaction by the removal of excess formaldehyde. Preliminary experiments indicated that such inactivated virus protein could be partially reactivated by incubation with dimethyldihydroresorcinol[2] or with histidine,[3] or by dialysis at pH 3 or at pH 7. Dialysis at pH 3 gave the best results and, since very little change occurred after the third day, a 3-day period of dialysis was adopted as the reactivation procedure.

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* The details of the work are contained in a paper to be submitted to *The Journal of Biological Chemistry.*

[1] Stanley, W. M., *Science,* 1936, **83**, 626.

[2] Wadsworth, A., and Pangborn, M. C., *J. Biol. Chem.,* 1936, **116**, 423.

The dialyzed solutions of inactivated and of reactivated virus protein were adjusted to M/10 phosphate (pH 7), analyzed for total nitrogen, and diluted with M/10 phosphate to concentrations suitable for inoculating against controls containing 10^{-5} or 10^{-6} gm. of active virus protein per cc.

Nicotiana glutinosa L. was chosen as test plant and the half-leaf method of inoculation was used,[4] with the exception that whole leaves were inoculated in tests for complete or nearly complete inactivation. The relative activities were interpreted after comparison with dilution curves of active virus and of mixtures of active and of inactive virus proteins. Inactive protein was found to reduce the lesion count by 40 to 50% when present at a concentration of 10^{-2} gm./cc., but had very little effect at 10^{-3} gm./cc. This reduction was no greater than that caused by comparable amounts of hydrogen peroxide-inactivated virus protein or of egg albumin. The preparations used for inoculation contained less than 10^{-5} gm./cc. of free formaldehyde, and several tests showed that this amount of free formaldehyde has no effect on activity and that concentrations as high as 10^{-3} gm./cc. have very little if any effect. The inactivation by formaldehyde can not be due to an aggregation of particles, for the formolized virus protein has about the same sedimentation constant[5] and exhibits approximately the same amount of stream double refraction as does active virus protein. Inactivation by means of formaldehyde causes a decrease in amino-nitrogen, as evidenced by Van Slyke gasometric determinations and by colorimetric estimations using ninhydrin. Inactivation is also accompanied by a decrease in the number of the groups that react with Folin's phenol reagent. It is probable that the latter are the indole nuclei of tryptophane. That formaldehyde is actually bound to the protein is indicated by the fact that, even after many days of dialysis, the formolized proteins give a strong Rosenheim-Acree test upon addition of H_2SO_4 and an oxidizing agent. These results, together with the fact that during the inactivation of about 99% of the virus protein the rate approximates that of a monomolecular reaction, demonstrate that the inactivation is not due to the toxicity of free formaldehyde or of the protein-formaldehyde complex and that it probably results from the blocking of amino groups, indole nuclei, or both.

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[3] Holden, H. F., and Freeman, M., *Austral. J. Biol. and Med. Sci.*, 1931, **8**, 189.

[4] Loring, H. S., *J. Biol. Chem.*, 1937, **121**, 637.

[5] Wyckoff, R. W. G., Biscoe, J., and Stanley, W. M., *J. Biol. Chem.*, 1937, **117**, 57.

When the reaction was stopped after suitable periods of time, preparations retaining approximately 10%, 1%, and 0.1% of their original activity were secured. These preparations following reactivation were found to possess approximately 20%, 10%, and 1%, respectively, of their original activity. Preparations that were inactive when inoculated at a concentration of 10^{-3} gm./cc. were appreciably infectious at 10^{-3} gm./cc. after reactivation. Varying the concentration of formaldehyde or lowering the pH did not significantly alter the amount of reactivation obtainable. The data indicate that two simultaneous reactions occur, one reversible and the other irreversible. The ten-fold increase in activity that may be obtained by the reactivation process is definite and is reproducible at will. The reactivation can be demonstrated by a comparison of the activity of an inactivated sample with that of the corresponding reactivated sample of the same protein concentration and can be measured accurately by a comparison of each at suitable dilutions against a common control. The results of several experiments agree quite closely. The amount of reactivation is many times greater than can be attributed to experimental error, to a toxic action of inactive protein, or to the removal of any remaining traces of free formaldehyde by the prolonged dialysis. Evidence that reactivation is accompanied by an increase in amino-nitrogen and in groups that react with the phenol reagent was obtained by colorimetric estimation.

The demonstration that the addition of formaldehyde to the virus protein results in a simultaneous decrease of activity, of amino groups and of reducing groups, and that under conditions favorable for the removal of formaldehyde the virus activity is regained and the number of such groups increases indicates that certain of these groups play at least a partial rôle in the structure necessary for virus activity. It should be noted that the reactivation of formolized virus differs from that which may be secured following inactivation with safranine[6, 7] or with the salts of heavy metals,[8, 9] for in the latter instances the complexes are insoluble and the possibility that they are non-infectious because of toxicity has not been excluded. In con-

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[6] Vinson, C. G., and Petre, A. W., *Bot. Gaz.*, 1929, **87**, 14.

[7] Krueger, A. P., and Baldwin, D. M., *J. Infect. Dis.*, 1935, **57**, 207.

[8] Stanley, W. M., *Phytopath.*, 1935, **25**, 899.

[9] Went, J. C., *Phytopath. Z.*, 1937, **10**, 480.

trast, the formolized virus protein is soluble, and it has been proved that the inactivation is not due to toxicity. The inactivation by means of formaldehyde, the subsequent reactivation, and the condition of certain groups in each case can best be interpreted by means of familiar chemical reactions and are direct experimental evidence that virus activity is a specific property of the protein.

9814

Sulfanilamide and Prontosil in the Treatment of Canine Distemper.*

VIRGINIA C. DICKERSON AND LEON F. WHITNEY. (Introduced by H. G. Barbour.)

From the Laboratory of Pharmacology and Toxicology, Yale University School of Medicine.

Sulfanilamide and prontosil have been found effective in otherwise apparently fatal cases of infection with meningococci[1] and hemolytic streptococci,[2, 3, 4] as well as in gonococci[5] infections, but curative value has not been shown in cases of infection with filtrable viri or with bacilli. It is now well established that canine distemper is caused by a filtrable virus.[6] The naturally occurring disease is, however, invariably complicated by infection with *B. bronchisepticus* and frequently with other secondary invaders. These greatly increase the mortality in distemper, and might be susceptible to the influence of sulfanilamide and its derivatives. We undertook to test this hypothesis in a naturally occurring epizoötic of distemper at the Whitney Kennels, Orange, Connecticut.

All the dogs exhibited typical distemper symptoms, including temperature curves. Moreover, two ferrets, injected with a suspension of the spleens of dogs which died during the epizoötic, developed severe distemper in 9 days; while 7 young dogs of known distemper-free history, vaccinated with a formolized suspension of the same spleens, have remained immune to distemper during a subsequent well-controlled exposure in other kennels.

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* This investigation was made possible through support given by the W. E. Weiss Fellowship Fund of the Winthrop Chemical Company.

[1] Schwentker, F. F., Gelman, S., and Long, P. H., *J. Am. Med. Assn.*, 1937, **108**, 1407.

[2] Mellon, R. R., Cross, P., and Cooper, F. B., *J. Am. Med. Assn.*, 1937, **108**, 1858.

[3] Colebrook, L., and Kenny, M., *Lancet*, 1936, **2**, 1319.

[4] Long, P. H., and Bliss, E. A., *Arch. Surg.*, 1937, **34**, 351.

[5] Dees, J. E., and Colston, J. A. C., *J. Am. Med. Assn.*, 1937, **108**, 1855.

[6] Laidlaw, P. P., and Dunkin, G. W., *J. Comp. Path. and Therap.*, 1926, **39**, 222.

Our experimental treatment was begun 6 days after distemper was first noted in the kennel, and a number of bloodhounds had already succumbed. Twenty-nine animals in various stages of the disease were divided into 4 groups as comparable for the purpose as possible. Four dogs received sulfanilamide, 9 prontosil, 4 both drugs, and 12 dogs served as untreated controls. Prontosil was injected subcutaneously as a 2.5% solution in 0.6% sodium chloride, while sulfanilamide was administered orally in the form of tablets of "Prontylin". In all cases the drugs were given 3 times daily in the dosages indicated below.

Neither prontosil nor sulfanilamide appeared to have any definite beneficial effect. One foxhound, obviously near death when treatment was begun, died 4 hours after prontosil, 50 mg. per kilo. Of the bloodhounds, a breed among which the mortality from distemper is exceptionally high, 2 with moderate fever receiving both drugs (prontosil 25 mg. per kilo and prontylin 50 mg. per kilo) survived for several weeks, but eventually died after treatment had been discontinued. The remaining 2 thus treated (in high fever and collapse respectively) and 3 untreated animals (2 with high and one with moderate fever) all died inside of 4 days. Two young "coonhounds" were given prontosil, 50 mg. per kilo, beginning 2 days after exposure to the disease, but like their 2 untreated littermates, died within 8 days. A treated (prontosil, 50 mg. per kilo) and an untreated Scotch terrier both survived. Of 2 cocker spaniels, while the untreated control died from some unknown cause one week after the return of the temperature to normal, the one receiving prontosil (50 mg. per kilo) survived.

Our most instructive results were obtained on a single litter of 13 young mongrels of high resistance. Beginning one day after exposure, 4 dogs were given prontosil (50 mg. per kilo), 4 were given sulfanilamide (33 mg. per kilo), and 5 received no treatment. A single dog from each group died within 8 days, and among the survivors there was no evidence that the disease was less severe in the treated than in the untreated control animals.

From the above study of 17 treated and 12 untreated dogs, no evidence has been found for the effectiveness of sulfanilamide or prontosil in the treatment of naturally occurring distemper. Our results emphasize the need for uniform stock for the study of dog diseases.

9815 P

Effect of Alcoholic Intoxication and Ether Anesthesia on Resistance to Pneumococcal Infection.

Kenneth L. Pickrell. (Introduced by Arnold R. Rich.)

From the Department of Pathology and Bacteriology, The Johns Hopkins University, Baltimore, Md.

There are numerous papers dealing with the effect of alcohol upon experimental infections, and it is rather generally agreed that infected intoxicated animals die sooner than similarly infected non-intoxicated controls. The manner in which alcohol lowers resistance has, however, not been satisfactorily explained. Numerous investigators have reported that alcohol inhibits phagocytosis but their experiments consisted in adding relatively enormous amounts of alcohol *in vitro* to the leucocyte-bacteria mixture. When small amounts of alcohol were used in such experiments there was no inhibition of phagocytosis.[1]

The present experiments were carried out in order to determine whether alcoholic intoxication lowers the resistance to pneumococcal infection, and if so, to attempt to discover the mechanism by which it exerts its effect. It was decided at the outset to study the effect of intoxication on well-immunized animals in order that any resistance-lowering potentiality that might be present would be put to as severe a test as possible.

Seventy-four rabbits have been used in the experiments here reported. In every experiment the procedure has been to immunize a group of rabbits by intravenous injection of Type I antipneumococcal serum, to intoxicate some of them, and then to infect all of them and, in addition, intoxicated and non-intoxicated non-immune controls, with the same dose of virulent Type I pneumococci endermally.

To produce intoxication 40 cc. of 20% ethyl alcohol was administered by stomach tube, smaller doses being repeated when necessary. The animals were maintained in a stuporous condition throughout the experiment. Blood cultures were made from all animals at intervals of several hours. The sites of infection were followed macroscopically throughout, and at the termination of the experiment they were removed for microscopic study. A typical protocol will be presented.

Twelve rabbits were highly immunized by the intravenous injection of 10 cc. of antipneumococcal serum. Six were then intoxicated with alcohol and all, and in addition normal control animals, were infected intracutaneously with 0.1 cc. of an 8-hour pneumococcal culture. The intoxicated non-immunized rabbits and the non-intoxicated non-immunized rabbits developed a positive blood culture at 5 hours, with 5 to 10 colonies per 10 drops of blood, and death from septicemia ensued in each instance within 18 hours. The intoxicated immunized rabbits showed a positive blood culture in 9 to 10 hours with 2 to 4 colonies, and death occurred from septicemia within 24 hours. The non-intoxicated immunized rabbits did not develop a positive blood culture, and survived.

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[1] Parkinson, P. R., *Lancet*, 1909, **2**, 1580.

No macroscopic lesion developed in any of the intoxicated animals in contrast to the edematous, hyperemic and purpuric lesion which extended to the belly-surface in the non-intoxicated non-immunized rabbits. Microscopic examination of the sites of infection revealed a dense leucocytic infiltration in all non-intoxicated animals. In the intoxicated rabbits, in sharp contrast, there was practically no leucocytic emigration and bacteria were present in swarms in the tissues in the immunized as well as in the non-immunized group. In the non-intoxicated immunized animals there was an abundant leucocytic exudate and no bacteria could be found in the sections after 9 hours.

Experiments in which the bacteria were introduced into the lung by way of the trachea gave results quite like those of the intracutaneous experiments.

Experiments of the same type carried out on immunized rabbits kept under ether-anesthesia gave precisely the same results as those described above in the case of the intoxicated immunized animals.

Since the temperatures of the intoxicated rabbits fall to a level as low as 97° F., a series was carried out to control this factor. It was found that lowering the body temperature to the level of that of the intoxicated rabbits, by keeping rabbits flat on their backs, did not inhibit leucocytic emigration and did not affect their immunity.

Blood counts showed that the scarcity of leucocytes at the infected site in the intoxicated animals could not be attributed to leucopenia. The effect of intoxication upon the activity of the phagocytes was therefore investigated in 24 animals. Five cc. of an aleuronat-suspension was injected into the pleural cavity and 18 hours later, when there was an abundant polymorphonuclear exudate, half of the rabbits were intoxicated. After 2 hours' intoxication, 2 cc. of a concentrated culture of Type II, rough, avirulent, pneumococci was injected into the pleural cavity. Smears of the pleural exudates made 45 minutes later showed abundant phagocytosis, and there was no difference in the phagocytic index between the normal and the intoxicated animals. Analyses at this time showed that the alcoholic content of the blood was never above 40 mg. higher than that of the pleural exudate, and both were as high or higher than the figures given in the literature for the blood level during human intoxication.

Differential counts in vital preparations of blood from both rabbits and patients in alcoholic stupor revealed no difference in the number of non-motile cells as compared with normals.

Conclusions. Alcoholic intoxication maintained at the point of stupor destroys resistance to pneumococcal infection in the rabbit. Even in animals rendered highly immune by the intravenous injection of antipneumococcal serum, intoxication deprives them of their immunity. The loss of resistance to the infection is due to the fact that intoxication profoundly inhibits the vascular inflammatory response as long as intoxication is maintained. Leucocytic emigration at the site of infection is negligible and the bacteria, therefore, proliferate uninterruptedly. Similar experiments show that ether-anesthesia has as marked an inhibitory effect on the inflammatory response as has alcoholic intoxication, and produces as marked a loss of resistance to infection.

9816

Pyridium as a Source of Interference in Vitamin C Determinations.

CATHERINE F. GANNON AND TERESA MCGOVERN. (Introduced by Irving Wright.)

From the Department of Medicine, New York Post-Graduate Medical School and Hospital, Columbia University.

The method commonly employed for determination of vitamin C in the body fluids makes use of Tillmans'[1] indicator, 2,6 dichlorophenol indophenol. This is not specific for the vitamin but under controlled conditions is practically so. Substances normally present in urine, namely cysteine, glutathione, and ergothioneine,[2] may reduce the dye, but Abbasy and coworkers[3] have reported that the non-specific titer did not rise above 3 to 6 mg. per day for the average person. In many specimens from our patients, where vitamin C was excluded from the diet, total excretion of "vitamin C" varied from less than one to 4 mg. in 24-hour specimens. Such interference, therefore, seems to be unimportant in the majority of cases. We have found, however, that Pyridium, (phenylazo-diamino-pyridine hydrochloride), a commonly used medication for disorders of the urinary tract, may constitute a serious source of error in blood[4] and urine[5] determinations of the vitamin.

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[1] Tillmans, J., Hirsch, P., and Jackisch, J., *Z. f. Untersuch. d. Lebensmitt*, 1932, **63**, 241.

[2] Emmerie, A., and Van Eekelen, M., *Biochem. J.*, 1934, **28**, 1153.

In one instance, a patient's excretion following a test dose, 1000 mg. intravenously,[6] of crystalline ascorbic acid,* decreased daily from 94 mg., to 32 mg., to 4 mg., and the next day the result obtained was 137 mg. On that day, however, 6 Pyridium tablets had been given in treatment of cystitis unrelated to the hypovitaminosis. The urine was diluted and titrated, and a distinct endpoint duplicated. This was the first apparent rise in excretion without increased intake in this subject. After the cystitis had disappeared a test dose of 1000 mg. orally and 6 Pyridium tablets were given for experimental purposes. The result obtained at a dilution of 1:40 was 385 mg., but after the specimen had stood in the refrigerator for 48 hours, the quantity present appeared to be 430 mg. In our experience the titer has increased on standing only in specimens which contained Pyridium.

A normal subject whose intake of vitamin C was regularly low and daily excretion less than 10 mg., apparently eliminated 40 mg. when given 4 Pyridium tablets. This exceeds the average for persons with adequate intake. After exclusion of foods rich in the vitamin for 4 days, a 24-hour urine contained 17 mg., and a blood specimen taken at 10:45 A. M. (following completion of the 24-hour sample of urine) 0.64 mg. per 100 ml. of plasma. At 11 A. M. two Pyridium tablets were taken. A blood specimen drawn 2 hours later showed highly colored plasma which, after protein precipitation, was pale yellow and gave a result of 0.77 mg. The 7 A. M. to 5 P. M. urine was highly colored, diluted 1:40 and indicated

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[3] Abbasy, M. A., Harris, L. J., Ray, S. N., and Marrack, J. R., *Lancet*, 1935, **2**, 1399.

[4] Farmer, C. J., and Abt, A., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625.

[5] Harris, L. J., and Ray, S. N., *Lancet*, 1935, **1**, 71.

[6] Wright, I. S., Lilienfeld, A., and MacLenathen, E., *Arch. Int. Med.*, 1937, **60**, 264.

* The crystalline ascorbic acid used in these studies was supplied through the kindness of Merck & Co., Inc., Rahway, N. J.

22 mg., while in the same period of the preceding day the excretion had been 6 mg. The 5 P. M. to 7 A. M. specimen was only slightly colored. When diluted 1:10 the result was 13 mg., as compared with 11 mg. during the same hours on the preceding day. The total 24-hour result was 35 mg., double that of the day before, the diet on both days being vitamin C-free. The usual dosage of 2 Pyridium tablets 3 times a day, which colors the urine very highly, was not given in this case, because of a complaint of nausea following the first two.

Pyridium was then added to urine specimens of known vitamin C content, and to standard solutions of crystalline ascorbic acid. In both the titer increased appreciably, as indicated in Table I.

TABLE I.

	Original Titer Ml. dichloro-phenolindophenol	Titer after Addition of Pyridium	
		Experimental Value	Corrected for dilution necessary to reduce color
Urine A	0.34	.44	(not diluted)
” B	0.21	.18	2.70 (dilution 15)
Standard A	1.13	.63	1.26 (” 2)
” B	1.24	.73	1.46 (” 2)
” C	1.36	.78	1.56 (” 2)

After addition of Pyridium, titration in the presence of its orange-amber color yielded results much higher than the original, and yet dilution to avoid color interference gave still higher values. In urine A, the addition of a dust-like particle of a powdered Pyridium tablet did not appear to alter the color of the specimen, but an increased titer resulted. In urine B, sufficient Pyridium was added to match the concentration in a specimen from a patient receiving this medication, and although practically colorless after being diluted 15 times, the titer was very close to that of the undiluted Pyridium-free specimen. Minute particles introduced into the standard solutions of ascorbic acid gave a very pale color which on dilution with an equal volume of water was barely perceptible. In all cases, however, the addition of Pyridium resulted in an increase of the titer with dichlorophenol indophenol.

The inability to obtain satisfactory results was noted in urine examinations as late as 3 days after Pyridium had been taken. Since varied efforts to remove Pyridium without loss of some of the vitamin C were unsuccessful, it would appear inadvisable to do this test by the present method on a patient who has had Pyridium medication within 5 days. Its presence may explain some apparently high ascorbic acid values unexpected from knowledge of the dietary of the individual.

In a study of the nutritional state of an individual in regard to vitamin C, many factors enter into an evaluation of analyses of the body fluids. Subnormal urinary values may sometimes be occasioned by loss in the sweat,[7] or, in certain manifestations of kidney disease, by retention in the blood.[8] The administration of salicylates[9] has been reported to accelerate the excretion of the vitamin, while the metabolic demands of fever, particularly in pneumonia[10] and tuberculosis,[11] may be responsible for a diminished output. That Pyridium medication may cause erroneously high vitamin C results is a possibility that should be considered.

9817

Progression of Different Nasally Instilled Viruses along Different Nervous Pathways in the Same Host.

ALBERT B. SABIN.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

In recent years much evidence has been accumulated to indicate that following nasal *instillation* many neurotropic viruses invade the central nervous system (CNS) by the olfactory pathway, and controversy has centered on whether these viruses progress along the perineural spaces or within the processes of the olfactory neurons themselves. Interruption of the olfactory pathway by surgical means has been shown to be capable of preventing poliomyelitis in monkeys induced by nasal instillation of the virus, in spite of the fact that all the other nerves connected with the nasal mucosa remain intact.[1] Similar interruption of the olfactory pathway in rabbits did not, however, prevent the encephalitis which follows the nasal instillation of herpetic virus,[2] and tests for virus during the incubationary period of the disease in normal, unoperated rabbits showed that it was present in the Gasserian ganglia but not in the olfactory bulbs, suggesting that invasion occurred along the 5th nerve.[3] It appeared, therefore, that either conditions were dissimilar in the rabbit and monkey or that different viruses behaved differently. To elucidate this question it was necessary either to vary the virus and keep the host constant or to vary the host and keep the virus constant; it proved easier to do the former.

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[7] Lilienfeld, A., Wright, I. S., and MacLenathen, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 184.

[8] Wright, I. S., and MacLenathen, E., PROC. SOC. EXP. BIOL. AND MED., in press.

[9] Daniels, A. L., and Everson, G. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 20.

[10] Bullowa, J. G. M., Rothstein, I. A., Ratish, H. D., and Harde, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 1.

[11] Martin, G. J., and Heise, F. H., *Am. J. Dig. Dis. and Nutr.*, 1937, **4**, 368.

[1] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728; Brodie, M., and Elvidge, A. R., *Science*, 1934, **79**, 235; Lennette, E. H., and Hudson, N. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1444; Howe, H. A., and Ecke, R. S., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 125; Gordon, F. B., and Lennette, E. H., *J. Bact.*, 1938, **35**, 43.

The viruses of vesicular stomatitis (V.S.), eastern equine encephalomyelitis (E.E.E.), and pseudorabies (the last having been shown to belong to the same "generic" group as herpetic and B virus[4]) were therefore studied in 15-day-old mice (Rockefeller Institute strain). Previous experiments on such mice have shown that following nasal instillation, V.S. virus is found in the olfactory bulbs before it can be detected in the Gasserian ganglia or pons.[5] It is necessary to stress that the nasal instillations were carried out without any mechanical injury to the mucosa, the mice being allowed to aspirate small drops of viral suspension placed on their nostrils. It should also be pointed out that with pseudorabic virus (centrifuged 10% suspension of rabbit-brain in broth) only half the number of mice receiving such *instillations* succumbed (first sign being a persistent and violent scratching of the nose and side of the face), while all mice receiving the same or a smaller dose by subcutaneous, intramuscular, or intraocular *injection* succumbed. Mice of this age were thus found to be much less susceptible to nasal instillation with pseudorabic virus than with E.E.E. or V.S. In the present investigation the pathological method already described in a study on V.S. virus[6] could be used to obtain an indication as to whether the other nervous pathways connected with the nasal mucosa are involved. Partial, serial, transverse sections were taken of the entire skull (without the lower jaw) with all the cranial nerve and autonomic ganglia (except the submaxillary) and superior cervical sympathetic ganglia *in situ,* and also of representative regions of the spinal cord. With the V.S. virus (mice succumbing in 4 to 5 days) and E.E.E. virus (2 to 3 days), the lesions which permitted the mapping of viral progression were those of neuronal necrosis, while with pseudorabic virus (about 54 hours), there was no neuronal necrosis but striking and unmistakable acidophilic, intranuclear inclusions served to indicate the path of the virus.

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[2] Levaditi, C., Hornus, G., and Haber, P., *Ann. Inst. Pasteur,* 1935, **54**, 389.

[3] Levaditi, C., and Haber, P., *Compt. rend. Soc. biol.,* 1935, **119**, 21.

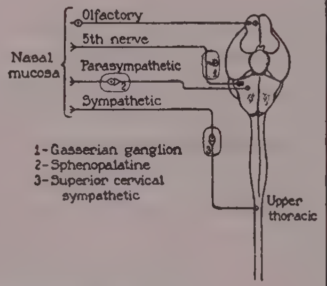
[4] Sabin, A. B., *Brit. J. Exp. Path.,* 1934, **15**, 372.

[5] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.,* 1937, **66**, 15.

[6] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.,* 1938, **67**, 201.

It is necessary to recall here the chief nervous pathways connected with the nasal mucosa and the situation of the cell-bodies of the neurons of the first and second orders (Fig. 1): (1) the olfactory pathway with the cell-body of the primary neuron in the olfactory mucosa and its *axon* coming into relation with the dendrites of the secondary neuron in the bulbs; (2) the fibers of the 5th nerve whose cell-bodies in the Gasserian ganglia synapse with the neurons of the sensory nucleus of the trigeminus in the medulla; (3) the sympathetic fibers whose cell-bodies in the superior cervical sympathetic ganglia are in synaptic relation with the neurons in the lateral horn of the lower cervical or upper thoracic region of the spinal cord; and (4) the bulbar autonomic ("parasympathetic") fibers whose cell-bodies in the sphenopalatine ganglia synapse with neurons situated in the region of the 7th nerve nuclei in the medulla.

Diagrammatic presentation of nerve supply of nasal mucosa and situation of neurons of first and second order in the nervous system of the mouse.



Nervous pathways selected by three different viruses in invading the central nervous system after nasal instillation in the mouse.

Nervous pathway	Virus		
	Vesicular stomatitis	Equine encephalomyelitis	Pseudo-rabies
Olfactory	+	+	−
5th nerve	−	−	+
Sympathetic	−	−	+
Bulbar autonomic (Para-sympathetic)	−	−	+

+ Lesions present along pathway
− " absent " "

FIG. 1.

It has already been reported that following nasal instillation in mice, the viruses of V.S.[6] and E.E.E.[7] invade the CNS along the olfactory pathway. In the present study there was no evidence of progression (neither neuronal necrosis nor inclusions) along the 5th nerve, sympathetic, or parasympathetic routes. With pseudorabic virus, however, the reverse was true; specific viral lesions, *i. e.*, characteristic intranuclear inclusions, were abundant in the Gasserian ganglia and sensory nuclei of the 5th nerve, in the sphenopalatine ganglia and region of the 7th nerve nuclei in the medulla, in the superior cervical sympathetic ganglia and the lateral horn of the lower cervical or upper thoracic regions of the spinal cord, but nowhere along the course of the olfactory pathway. In a trial test on an animal sacrificed at the onset of nervous signs (which is about 6 hours before death) virus was found (by subinoculation in rabbits) in the Gasserian ganglia and medulla but not in the olfactory bulbs, indicating that in this case the distribution of virus coincided with the observed pathological changes. The following observations support the assumption that the pseudorabic lesions in these structures are the result of the neural progression of virus from the nose and not of any special affinity which virus in the blood might have for them: (a) after injection into the leg muscles there is abundant virus in the blood but no lesions in any of these structures; (b) after nasal instillation of the virus the lesions have been unilateral (in the mice studied thus far), on the same side in all the involved pathways, and none was found in ganglia having no connection with the nasal mucosa. A more detailed description of the localization of lesions in mice after injection of pseudorabic virus by various routes will be given in a future communication, in which it will also be shown that after intraocular (vitreous) injection this virus utilizes pathways (including sympathetic) not employed by the viruses of V.S. and E.E.E. Perhaps the first evidence of the centripetal progression of a virus along the cranial sympathetic system has been obtained in the present study.

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[7] Sabin, A. B., and Olitsky, P. K., *Am. J. Path.*, 1937, **13**, 615 (Abstract).

It appears clear, therefore, that the facility with which certain neurotropic viruses (*i. e.,* viruses which attack nerve-cells and may or may not be able to attack other types of tissue as well) invade the CNS after nasal instillation is dependent neither upon any supposed connection between the exterior of the nasal mucosa and the subarachnoid space through the olfactory perineural spaces, nor apparently even upon the special position of the cell-bodies of the primary olfactory neurons with their capacity to take up certain instilled substances,[8] but rather upon the special affinity between certain viruses and certain types of cells. In this connection an explanation is required of some recent observations on the fate of a number of viruses instilled into the nose.[8] It was stated that the viruses of St. Louis encephalitis, rabies and louping-ill could not be detected in the "olfactory area" of the brain in less than 24 hours after nasal instillation in mice (the same was found to be true of V.S. virus[5]), while E.E.E. virus was demonstrated within 2 to 5 minutes although not at 6, 8, and 10 minutes or at 6 hours. On the basis of these data, it was assumed that the former 3 or 4 viruses which were called "neurotropic" invaded the nervous system differently from the E.E.E. virus which was termed "pantropic". E.E.E. virus, however, is just as neurotropic as the others and its capacity to multiply in non-nervous tissue elements of mice is shared to a certain extent by the St. Louis encephalitis,[9] louping-ill,[10] and V.S. viruses.[11] It appeared probable that this singular result with the E.E.E. virus may have been due to the fact that a given dilution of mouse-brain suspension contains more infective units of this virus than any of the other four; the amount of E.E.E. virus instilled may be estimated at 20 million to 200 million minimal cerebral lethal doses (M.C.L.D.), while the dosage of the other viruses probably ranged between 100,000 and 2 million M.C.L.D. Also it is conceivable that with the larger amount of virus in the nares in the first few minutes (nasally instilled virus has been shown to be rapidly washed away[5, 12]) it might be possible to pull up one or more infective units in taking out the olfactory bulbs. An experiment designed to elucidate this question revealed the following: (a) that when the olfactory bulbs were taken out without precautions to avoid pulling, a trace of virus was found in them (within 2 to 4 minutes) when 10 million M.C.L.D. of E.E.E. virus were instilled but not 1 million; (b) that 200 million M.C.L.D of E.E.E. virus could be instilled without any of it being demonstrable in the olfactory bulbs when they were removed by first being severed from

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[8] Rake, G., *J. Exp. Med.,* 1937, **65**, 303.

TABLE I.

Does Equine Encephalomyelitic Virus Invade the Brain Within a Few Minutes After Nasal Instillation?

Mice killed at 2 minutes, exsanguinated and nerve tissue removed from cranial cavity within 4 minutes.

Method of removing olfactory bulbs	Dilution of virus and No. of M.C.L.D. instilled	Mouse No.	Test for virus No. of mice injected	No. of mice dead
Special precautions to prevent pulling	1:5	1	2	0
	(200,000,000)	2	2	0
		3	2	0
		4	2	0
Olfactory bulbs pulled	1:100	5	2	1*
	(10,000,000)	6	2	0
	1:1,000	7	2	0
	(1,000,000)	8	2	0

[9] Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1936, **63**, 433.

[10] Fite, G. L., and Webster, L. T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 695.

[11] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1937, **66**, 35.

[12] Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1938, **67**, 229.

*Virus proved to be cause of death.

the rest of the brain and then from the olfactory nerves without injury to the cribriform plate and lifted out without any pull on structures in the nasal mucosa (Table I). It would appear, therefore, that there is as yet no evidence that nasally instilled viruses invade the CNS by any direct, open space; the available data seem to point rather to progression along nerve cells and their processes.

Conclusions. Different neurotropic viruses instilled into the nose of the same host (mouse) can select different nervous pathways for invading the central nervous system. The viruses of vesicular stomatitis and equine encephalomyelitis were shown to use the olfactory pathway but not the trigeminus, sympathetic, or parasympathetic pathways, while pseudorabic virus invaded along the latter three routes and not along the olfactory.

9818

Inhibition of Streptococcal Hemolysin by Sulfonamide Compounds.

PAUL GROSS, FRANK B. COOPER AND MARION LEWIS. (Introduced R. R. Mellon.)

From the Institute of Pathology, the Western Pennsylvania Hospital, Pittsburgh, Pa.

In vitro experiments, in which prontosil I* appeared to inhibit streptococcal hemolysin and leukocidin, have led Levaditi and Vaisman[1] to believe that certain sulfonamide compounds exert an antitoxic action.

The present report concerns the effect of sulfanilamide and other substances upon streptococcal hemolysin, and also the effect of sulfanilamide upon staphylococcal hemolysin.

Hemolysin was prepared by growing beta hemolytic streptococcus strain C 203 in 2% neopeptone beef broth of pH 7.6-7.8 for 16-18 hours and removing most of the bacteria by centrifugation.

In some experiments this hemotoxin was incubated with various amounts of sulfanilamide† (50 mg. per 100 cc. up to saturation) for 30 minutes at 37.5°C. The untreated toxin and the sulfanilamide-toxin were then titrated with washed rabbit erythrocytes. An incubation period of 45 minutes at 37.5°C. was allowed before the tubes were centrifuged and readings made.

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* **Prontosil I (4-sulfonamide-2′,4′-diaminoazobenzol) and prontosil II (disodium salt of 4-sulfamidophenyl-2′-azo-7′acetylamino-1′hydroxynaphthalene-3′,6′ disulfonic acid) kindly supplied by Winthrop Chemical Co., N. Y.**

[1] Levaditi, C., and Vaisman, A., *Compt. rend. Soc. de biol.*, 1935, **120**, 1077.

† **Kindly supplied by E. R. Squibb & Sons, New York.**

The addition of sulfanilamide to streptococcal hemolysin up to the point of saturation did not remove the hemolytic effect of the toxin. However, a very slight inhibitory action of the sulfanilamide was demonstrable provided a careful and close titration was done (Table I).

TABLE I.

Comparison of Hemolytic Action of Untreated Streptococcal Hemolysin with That Incubated with Sulfanilamide.

				Hemolysis	
Toxin A 1:10 cc.*	Toxin B 1:10 cc.*	2% Rabbit R.B.C. cc.	Saline cc.	Normal Toxin	Toxin saturated with sulfanilamide
.3		.1	.3	+	0
.4		.1	.2	++	+
.5		.1	.1	+++	++
.6		.1	.0	++++	+++
	.1	.1	.4	0	0
	.2	.1	.3	++++	+
	.3	.1	.2	++++	++++
	.4	.1	.1	++++	++++

*C 203 Toxin.

This slight inhibitory effect of sulfanilamide was apparent in numerous titrations only when the amounts of toxin corresponded to one minimal hemolytic dose or less. It was observed that if amounts of toxin in excess of one minimal hemolytic dose were used, complete hemolysis was obtained regardless of the amount of sulfanilamide present.

The effects of prontosil I and II* as well as of other substances upon streptococcal hemolysin were similarly investigated. Toxin samples containing the following: 0.4% prontosil I, 0.4% prontosil II, 0.2% eosin, 0.2% mercuric chloride, 0.2% phenol, and 0.4% sodium carbonate were incubated for 30 minutes at 37.5°C. and then titrated along with untreated toxin using 4% rabbit erythrocytes. After 45 minutes of incubation at 37.5°C. the tubes were centrifugated and readings taken.

Marked inhibition of hemolysis occurred with prontosil II, eosin, mercuric chloride, phenol, and sodium carbonate but the hemolytic titre of toxin containing 0.4% prontosil I was identical to that of untreated toxin (Table II).

TABLE II.
Comparison of Hemolytic Action of Untreated Streptococcal Hemolysin with That Incubated with Various Chemicals.

			Hemolysis						
Toxin Mixture* cc.	4% Rabbit R.B.C. cc.	Saline cc.	Toxin Alone	Prontosil I 0.4%	Prontosil II 0.4%	Eosin 0.2%	$HgCl_2$ 0.2%	Phenol 0.2%	Na_2CO_3 0.4%
.03	.1	.47	0	0	0	0	0	0	0
.06	.1	.44	+	+	0	0	0	0	+
.09	.1	.41	++	++	0	0	0	0	+
.12	.1	.38	+++	+++	0	0	0	0	+
.15	.1	.35	++++	++++	+	0	0	0	+
.18	.1	.32	++++	++++	+	0	0	0	+

*To 2.0 cc. portions of C 203 toxin 0.5 cc. saline and 5 or 10 mg. of the various inhibitory substances were added to make 0.2 or 0.4% solutions as indicated. These portions were then incubated for 30 minutes at 37.5°C. prior to titration.

Normal rabbit serum was found capable of inhibiting hemolysis by streptococcal hemotoxin.[2] This inhibition was not enhanced by the addition of sulfanilamide. Identical results were obtained with sera from rabbits which had received sulfanilamide orally (1.0 to 1.7 gm. per kilo) 4 hours prior to bleeding. These sera, containing 5 to 56 mg. of sulfanilamide per 100 cc., were no more inhibitory than the sera taken from the same animals prior to the administration of the drug.

The inhibitory effect of the sera was not destroyed by heating for 30 minutes at 55 to 61°C., and was found to be more pronounced in the case of a preliminary incubation of erythrocytes with serum than in the case of a preliminary incubation of toxin with serum.

Experiments with staphylococcal hemolysin† also indicated a very slight inhibitory effect when the hemotoxin was given a preliminary incubation with sulfanilamide (300 mg. per 100 cc.). This inhibitory effect could be observed only at and below one hemolytic dose.

A number of repeated tests with hypotonic saline indicated that the fragility of rabbit erythrocytes was not affected by the presence of sulfanilamide.

The demonstration in these experiments that prontosil I does not inhibit the hemolytic activity of streptococcal hemotoxin is opposed to the findings of Levaditi and Vaisman[1] and throws some doubt upon their assumption that sulfonamide compounds are antitoxic. Although a marked inhibitory effect upon the hemolytic activity of streptococcal hemotoxin was obtained with prontosil II and a very slight one with sulfanilamide, the assumption that this effect is due

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[2] Zinsser, H., and Bayne-Jones, S., *A Textbook of Bacteriology*, Seventh Edition, D. Appleton-Century Co., New York, 1935, p. 319.

TABLE III.

Comparison of Inhibitory Action of Untreated Serum (on Streptococcal Hemolysin) with That of Serum Containing Various Concentrations of Sulfanilamide.

C 203 Toxin cc.	Rabbit Serum cc.	4% Rabbit R.B.C. cc.	Saline cc.	Hemolysis					
				No Serum	Serum containing sulfanilamide mg. per 100 cc.				
					0	10	100	500	Saturated
.10	—	.1	.40	++++					
.15	.05	.1	.30		+	+	+	+	+
.15	.04	.1	.31		+	+	++	+	++
.15	.03	.1	.32		++	++	+++	+++	+++
.15	.02	.1	.33		+++	++++	++++	+++	+++
.15	.01	.1	.34		++++	++++	++++	++++	++++

to antitoxic action does not appear warranted because other substances such as normal serum (heated to 61°C.), eosin, phenol, mercuric chloride, and sodium carbonate have a similar effect.

Conclusions. Sulfanilamide causes a slight, almost negligible, inhibition of the hemolytic activity of streptococcal and staphylococcal hemotoxin. More marked inhibition of streptococcal hemolysin is produced by prontosil II, normal rabbit serum, rabbit serum heated to 61°C., eosin, phenol, mercuric chloride, and sodium carbonate. The administration of sulfanilamide *in vivo* or its addition to serum *in vitro* does not enhance the inhibitory effect of rabbit serum upon streptococcal hemolysin. Prontosil I was found to have no inhibitory effect upon streptococcal hemolysin.

9819 P

Protective Effects of CaCl$_2$ Against Procaine Convulsions in Guinea Pigs.*

R. Beutner and G. P. Miley.

From the Department of Pharmacology, Hahnemann Medical College of Philadelphia.

In order to protect animals against the convulsions from high doses of procaine-HCl, an addition of CaCl$_2$ was found to be effective. Guinea pigs, which *rarely* succumb to procaine convulsions, are more suitable for such studies than rabbits, which were formerly used by Beutner, Prusmack, and Miller[1]; rabbits almost invariably die from procaine convulsions. Guinea pigs, however, can be injected repeatedly with convulsant doses. In the case of procaine, just as with various other previously observed convulsant drugs,[2] numerous observations are necessary to determine the average convulsant dose.

As controls, 55 guinea pigs received 159 injections of 100 mg. per kilo, as 10% solution, of procaine-HCl *alone.* This means that each of these 55 pigs was injected at least twice and some as many

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* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council of Pharmacy and Chemistry, American Medical Association.

[1] Beutner, Prusmack, and Miller, *J. Pharm. and Exp. Therap.*, 1936, **57**, 114.

[2] Lennox, Nelson, and Beetham, *Arch. Neurol. and Psych.*, 1929, **21**, 625; *Proc. Physiol. Soc. of Philadelphia*, 1937, **12**, 10.

as 5 times. Of these 159 injections 138 resulted in convulsions, an incidence of 86.7%. Only 2 of all these animals died.

Forty-eight guinea pigs received 139 injections of 100 mg. procaine-HCl and 100 mg. $CaCl_2$. These two salts were mixed before injection in a solution containing 10% of each by volume. Previous to the $CaCl_2$-procaine-injections the animals had all received one or several injections of procaine alone, the same animals being used which were used as controls. Only 19 convulsions occurred in this series of 139 procaine-$CaCl_2$ injections, an incidence of 14.6% as contrasted with the 86.7% incidence obtained after procaine alone.

If a mixture of procaine and $CaCl_2$ was injected into guinea pigs which had had *no* previous injection of any kind, a somewhat higher incidence of convulsions was seen. We used only 10 animals in this series and gave them 30 injections of 100 mg. procaine-HCl plus 100 mg. $CaCl_2$, mixed before the injection as described above. In this series 12 convulsions occurred, or an incidence of 40% as contrasted with 86.7% from procaine alone. The protective effect of $CaCl_2$ is therefore manifest.

No protective effect of $CaCl_2$ was observed if $CaCl_2$ was first injected and followed by procaine-HCl a few minutes later; 12 guinea pigs were used for these experiments; all except one had convulsions, whereupon the experiment was discontinued. It appears that a mixing of the procaine-HCl and the $CaCl_2$ before the injection is essential for the protective action of $CaCl_2$.

We also tried to obtain a protective action by stimulating the calcium metabolism of guinea pigs by feeding them the drug AT 10, highly concentrated vitamin D, along with a diet rich in calcium. But this did not influence the incidence of convulsions after procaine at all; it remained at least as high, *viz.*, 90%, as after procaine given to a normal guinea pig; 12 animals were used in this series.

Conclusion. $CaCl_2$ injected intramuscularly in a mixed solution with procaine-HCl protects guinea pigs against convulsions ordinarily produced by injection of procaine-HCl alone.

9820 P

Amino-Acids in Staple Foods. The Rôle of Cystine in Proteolytic Digestion.

FRANK A. CSONKA.

From the Protein and Nutrition Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture.

The author presented his views on the synthesis of amino-acids in the animal body at the Washington annual meeting of the Society of Biological Chemists in 1936 (unpublished) and concluded that cystine, contrary to the view held at that time, is a dispensable amino-acid. His working hypothesis was that sugar forming amino-acids are dispensable and non-sugar formers are indispensable, and also suggested that valine being a non-sugar former would prove to be an indispensable amino-acid. There are so many examples in the literature where the diet under investigation was supplemented with cystine which promptly converted it to a normal growth-producing one that a claim of cystine deficiency was readily granted. Johns and Finks[1] fed cooked navy bean meal supplemented with cystine to rats and obtained normal growth so that they concluded that cystine deficiency was the controlling factor in the bean diet. Mitchell and Smuts'[2] feeding experiments demonstrated clearly that by supplementing raw soybean meal with cystine the rats not only stayed alive but gained in weight. Csonka and Jones[3] stated, however, that there cannot be a quantitative deficiency in soybean meal when the defatted meal contains an average of 0.4% cystine. Regardless of the cystine content of the experimental diets of soybean (high) and navy bean (low) the addition of cystine produced growth. Since the dispensability of cystine to growth has been now demonstrated by Rose and coworkers[4] experimentally, we are forced to assume that supplementary cystine performs some physiological function other than correcting a nutritional deficiency. There is some justification for considering first that free ^{-}SH has an activating effect upon proteolytic enzymes and, second, that the oxidation-reduction properties of this cystine (or cysteine) may affect digestion favorably. The former creates an oxidizing medium which is conducive, according to Voegtlin, Maver, and Johnson,[5] to synthesis of proteins, while the latter imparts a reducing medium favorable to hydrolysis. It was found by the author that the addition of cystine or cysteine to navy bean meal has an accelerating effect on peptic and tryptic digestion *in vitro*. The acceleration of proteolytic enzyme activity is more pronounced with cysteine supplementation when the substrate is in the raw state; when the cooked bean meal is digested, cystine is found more effective. A quicker and higher degree of digestion may result in a better assimilation and utilization of the bean protein by the animal body, which results in increased body weight, the improvement noted in feeding experiments mentioned above.

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[1] Johns, C. O., and Finks, A. J., *J. Biol. Chem.*, 1920, **41**, 379.

[2] Mitchell, H. H., and Smuts, D. B., *J. Biol. Chem.*, 1932, **95**, 263.

[3] Csonka, F. A., and Jones, D. B., *J. Agric. Res.*, 1934, **49**, 279.

[4] Rose, W. C., *Science*, 1937, **86**, 298.

The cystine present in peptide linkage in bean seed proteins apparently is not in available form; free cystine is not formed by digestion with pepsin or trypsin. Acid hydrolysis of a pepsin digest showed that all the cystine present in the seed originally was accounted for, but acid hydrolysis of a trypsin digest showed no detectible cystine by Sullivan's method. These experiments were carried out on defatted soybean meal in 1934.

Autolysis of the raw bean meal in (pH 5) disodium citrate solution is also accelerated by the addition of H_2S, cysteine or cystine in the order mentioned, which suggests the presence of a plant pepsin in the navy bean seed.

TABLE I.

Digestion Experiments with Raw Navy Bean Meal. Incubation Temperature 38°C.

		Autolysis		Pepsin		Trypsin	
	Type of N	3 hr.	72 hr.	3 hr.	72 hr.	3 hr.	72 hr.
Raw navy bean meal	Amino-acid	1.47	4.41	4.2	5.6	11.2	15.4
	Polypeptide	0.63	3.99	0	3.0	3.8	7.6
50 mg. cysteine supplement	Amino-acid	0	2.94	7.0	8.4	9.8	19.6
	Polypeptide	1.05	4.61	3.6	8.2	10.2	10.4
50 mg. cystine supplement	Amino-acid	3.15	5.88	7.0	7.0	12.6	16.8
	Polypeptide	0	3.57	0.6	5.6	3.4	8.2
H_2S	Amino-acid	2.94	1.47				
	Polypeptide	0.21	4.83				

To show the magnitude of acceleration caused by cystine or cysteine supplementation, digestion experiments on raw navy bean are tabulated in a condensed form. In the pepsin digestion 20 cc. of 0.05 N HCl containing 0.2 gm. of pepsin (Merck) was added to 2 gm. of navy bean meal containing 75 mg. N., while in the trypsin experiments the 2 gm. navy bean meal was suspended in 20 cc. of disodium phosphate solution of pH 8.9 containing 0.2 gm. of trypsin (Fairchild's). Willstätter and Waldschmidt-Leitz's[6] titration method was used and the figures represent increases over those obtained at the beginning, expressed in cubic centimeters of 0.1, normalcy representing 1.4 mg. amino N per cc. In pepsin digestion changes in the polypeptide N and in trypsin digestion, changes in the amino-acid N served as a measure of proteolytic acceleration.

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[5] Voegtlin, C., Maver, M. E., and Johnson, J. M., *J. Pharm. and Exp. Therap.*, 1933, **48**, 241.

9821 P

Investigations on the Pathogenesis of Tetanus.

BERNARD ZUGER AND ULRICH FRIEDEMANN. (Introduced by Benjamin Kramer.)

From the Division of Bacteriology, Jewish Hospital of Brooklyn.

We have observed that 10 to 20 times more tetanus antitoxin given intravenously is required to protect guinea pigs and rabbits against a multiple lethal dose of the toxin injected intramuscularly than intravenously. Table I gives the results of a representative experiment.

TABLE I.

Comparison of the Amounts of Antitoxin Required to Protect Guinea Pigs against 10 Fatal Doses of Tetanus Toxin Injected Intramuscularly and Intravenously.

Tetanus Toxin—N. Y. City Board of Health Laboratories Lot 47 Vial C.
Antitoxin " " " " " " Lot 284.

Antitoxin 1 cc. intravenously, dilutions	Toxin 0.1 cc. 1:200			
	G.p. No.	Intramuscularly	G.p. No.	Intravenously
1:50	507	LT, partial		
1:100	506	LT, "		
1:200	505	LT, complete		
1:500	504	LT, "		
1:1000	503	LT, "		
1:2000	502	LT, GT *3		
1:4000	501	LT, GT *6		
1:5000			511	0
1:10,000			510	0
1:20,000			509	0
1:40,000			508	GT *5

LT—local tetanus.
GT—generalized tetanus.
0—no symptoms.
*—death, number following indicating day after injection.

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[6] Willstätter, R., and Waldschmidt-Leitz, E., *Berichte*, 1921, **54**, 2988.

In further experiments we found that free antitoxin was present in the blood of the intramuscularly-injected animal at the time of death. These observations, we believe, have an important bearing on the pathogenesis of tetanus. They show that the death of the animal cannot be due to toxin reaching other muscles or the central nervous system by way of the circulating blood.

After excluding the Danysz phenomenon as a factor in our results we were faced with the alternative that either, contrary to Abel's[1, 2, 3] conception, tetanus toxin reaches the central nervous system by way of the peripheral nerve, or that a secondary toxic substance is formed in the muscle which is not neutralized by antitoxin. The first substance we suspected was acetylcholine. This phase of the work is still going on.

In searching for this hypothetical substance, toxin and muscle were mixed *in vitro*. It was found that when injected intramuscularly, the "muscle toxin" was 4 to 8 times more lethal than the pure toxin and 8 times more effective in causing local tetanus. In Table II we present a protocol of one of the many experiments we have carried out with this phenomenon.

The mixture is not more toxic than the pure toxin when injected intravenously. We also found that irrespective of the toxicity,

TABLE II.

Comparison of Lethal Dose of Muscle-toxin and Plain Toxin.

Preparation of Muscle-toxin—5 gm. of muscle from rear extremity of exsanguinated guinea pig triturated with 2 cc. of tetanus toxin (Eli Lilly Company) in mortar with aid of 2 cc. of sterile sand. Mixture centrifuged at 2000 r.p.m. for 15 minutes. Dilutions made from supernate, which was usually slightly in excess of 2 cc.

1 cc. injected intramuscularly in calf region of rear leg. Dilutions:	G.p. No.	Muscle-toxin	G.p. No.	Plain Toxin
1:2,000	692	LT *2	30	LT *2
1:4,000	710	LT *2	545	LT, GT *3
1:8,000	715	LT, GT *3	616	LT, complete
1:16,000	731	LT, GT *4	631	0
1:32,000	803	LT, GT *7	648	0
1:64,000	711	LT, complete		
1:128,000	756	0		

LT—local tetanus.
GT—generalized tetanus.
0—no symptoms.
*—death, number following indicating day after injection.

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[1] Abel, John J., Evans, E. A., Jr., Hampil, B., and Lee, F. C., *Bull. Johns Hopkins Hosp.*, 1935, **56**, 84.

[2] Abel, J. J., Hampil, B., and Jonas, A. F., Jr., *Ibid.*, 1935, **56**, 317.

[3] Abel, J. J., and Hampil, B., *Ibid.*, 1935, **57**, 343.

equal amounts of muscle toxin and pure toxin were neutralized by equal amounts of antitoxin *in vitro* and *in vivo*. This shows that the hypothetical secondary substance is not present in the mixture of muscle and toxin, although this does not preclude its production in the living muscle. It is also clear that the amount of toxin in the mixture is not increased. The mechanism of the potentiation of the toxin by the muscle is still under investigation.

9822

Further Studies of Agar-slant Tissue Cultures of Typhus Rickettsiæ.*

HANS ZINSSER, H. WEI AND FLORENCE FITZPATRICK.

From the Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

In a preliminary note published in these PROCEEDINGS,[1] the writers described an agar-slant-tissue method for the cultivation of Rickettsiæ, both of the murine and of the classical types of typhus fever.

Further study of this method has led to modifications and standardizations which are reported herewith in order to facilitate its use in the hands of others. Preliminary experiments by Pinkerton with Rocky Mountain Spotted Fever virus indicate a possibility that the method may be successful with Rickettsiæ other than those of typhus.

The medium as now used differs from the original only in proportions of ingredients. We have found that results are most regular when the agar is prepared as follows:

A "Tyrode" solution of the ordinary formula is made up double strength throughout, except that only 1 gm. of bicarbonate of sodium is used per liter. The agar used is a Difco granular Bacto-agar.

A mixture of 150 cc. double strength Tyrode and 150 cc. of horse serum with 8 cc. of phenol red solution is filtered through a Seitz filter.† To the filtrate is added 150 cc. of 4% agar in distilled water, cooled to 45°C.

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*This work was supported in part by a grant from the Commonwealth Foundation.

[1] Zinsser, Hans, Wei, H., and FitzPatrick, Florence, PROC. SOC. EXP. BIOL. AND MED., 1937, 37, 604.

The mixture is immediately put into 6 x ¾ inch test tubes—not more than 8 cc. to each tube; the tubes are closed with rubber stoppers and can be kept in the refrigerator. The initial reaction should be pH 7.4 to 7.6. This point is very important.

The Tissue. For the tunica tissue of guinea pigs which was used in the early cultures, we have now been able to substitute mouse embryo tissue. This has the advantage of greater ease of sterile manipulation, of furnishing larger amounts of tissue at smaller expense and of greater regularity of results. In general, the smaller the embryos the better. When large embryos are used the liver had best be removed.

Inoculation. For subcultures, the infectious material from a preceding tube is placed into a short and wide sterile tube (the ordinary 60 cc. centrifuge tube), together with the fresh embryonic tissue and the two are minced together with a long handled pair of scissors. This material is then laid on the agar so that about one-third of the agar surface is covered. It is important to avoid piling up the tissue.

Growth is usually determinable after 4 days and is at its maximum in from 6 to 8 days.

The eventual objective of attempts to develop a simple method of Rickettsia cultivation was to obtain quantitatively adequate amounts of the European organisms for vaccine production. The X-rayed rat technique, which continues to furnish satisfactory quantities of Rickettsiæ of the murine type, has consistently failed with the European strain. There was thus no available source of this latter type sufficient in yield to permit homologous vaccination experiments other than the louse vaccine devised by Weigl.

The cultivation method here described appears to have solved this problem. (Fig. 1.) But it was of great importance to make sure that these cultures had not dissociated into avirulent and antigenically modified forms. For this reason animal experiments were carried out.

It may be stated that the European strain now carried on agar was isolated from an infected guinea pig on Maitland medium on November 9, 1936. It was carried through 33 Maitland genera-

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† Owing to the frequent alkalinity of the Seitz filter discs, we have been rapidly washing these discs with 3 changes of distilled water in a Petri dish and drying them overnight in the incubator before putting them into the holders for sterilization.

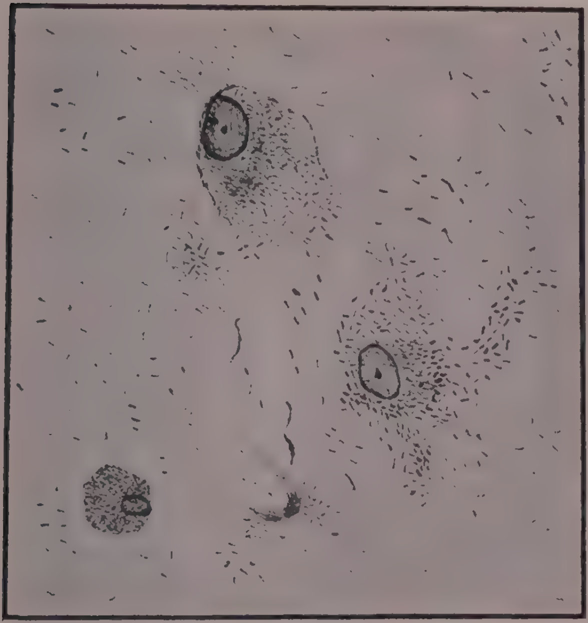

FIG. 1.

Smear on slide of Rickettsiae from 6-day agar tissue culture (mouse embryo tissue).

tions and transferred to agar tissue culture on November 8, 1937. It is now in its 12th agar generation, that is its 45th culture generation and has been out of the animal body for a period of over 15 months. With the 19th Maitland culture generation we were able to produce typical temperature reactions, specific immunity to passage virus and characteristic brain lesions in guinea pigs.

We have carried out a series of animal inoculations with agar cultures representing respectively the 5th, 6th, 7th, and 8th agar culture generations (*i. e.*, 38th to 41st generations outside the animal body). In all of these animals temperature reactions developed which, in most cases, differed from the passage virus controls only in that the incubation times were shortened to 4 or 5 days instead of the usual 6 to 9 days. In all of these animals subsequent inoculation of passage virus demonstrated specific immunity. (Fig. 2.) In a few animals in which cultures older than 10 days were used the reactions were mild—but subsequent immunity tests were nevertheless positive. Some of these animals showed, at an early stage of the fever, the scrotal swelling not infrequently observed in European

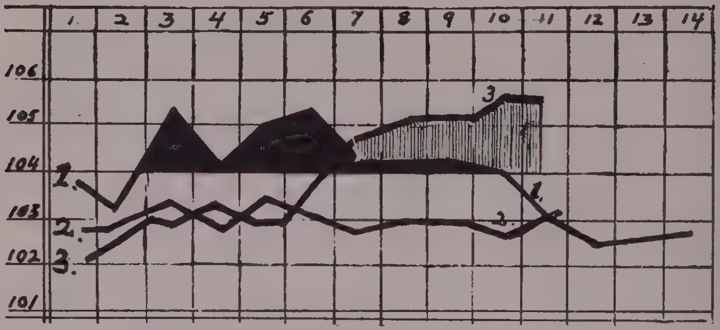

FIG. 2.

1. Temperature curve of guinea pig inoculated intraperitoneally with organisms from 7th generation agar culture. This agar strain started from 33d generation of classical European typhus Rickettsiæ on Maitland flasks. The inoculum therefore represents 40th culture generation isolated from an infected guinea pig on Nov. 9th, 1936. Culture inoculation on Jan. 3d, 1938.

2. Temperature curve of same guinea pig inoculated intraperitoneally on Jan. 31st, 1938, with passage virus, blood and brain of same strain maintained in guinea pigs.

3. One of two passage virus controls of immunity test as above.

passage virus infections when heavy inoculation is used. In one animal a testicle was removed on the fourth day when the temperature had risen to 105.8. Examination of the tunica showed a few Rickettsiæ and inoculation of tunica material into another guinea pig resulted in a severe and characteristic typhus reaction. The original animal recovered, defervescing on the 12th day and proved immune on reinoculation with properly controlled passage virus.

It is thus clear that the Rickettsiæ of the European type cultivated for 15 months through 33 Maitland generations and 8 subsequent generations on agar-tissue cultures have lost neither their characteristic infectiousness nor their capacity for specific immunization.

This encourages the hope that the method may furnish a satisfactory source of the European type of Rickettsiæ *(Rickettsiœ prowaceki)* for vaccine production.

9823

Effect of Nicotinic Acid on Increased Porphyrinuria Occurring in Seven Painters.

E. S. Gross, Y. Sasaki and T. D. Spies.

From the Department of Internal Medicine, University of Cincinnati.

Recently, Spies, Gross and Sasaki[1] demonstrated that the increased porphyrinuria occurring in pellagra and certain other diseases decreases rapidly following the administration of nicotinic acid. In view of these observations, it seemed important to determine the effect of this substance on increased porphyrinuria due to some other cause. Previous work[2] has shown that lead poisoning may be accompanied by increased porphyrinuria. Since many paints contain lead, it seemed likely that men who had been painters for a number of years might have increased porphyrinuria. Seven painters, who had been exposed to lead for a period of at least 15 years were, therefore, selected for study.

A group of 45 medical students served as controls for the degree of porphyrinuria one might expect in normal adult males. Samples of urine from each member of this control group were collected on the same day and were tested for increased porphyrinuria.* A sample was reported positive only if the color was definitely pink; no color or traces of color were considered to be negative tests for increased porphyrinuria. Of the 45 samples of urine, 22 showed no color, ten showed only a trace of color, and 13 showed a definite pink color. Since subsequent tests on the urine from this latter group showed no color or only traces of color, with the exception of one case, the authors believe that the ingestion of alcohol and dietary irregularities might account for the slightly increased porphyrinuria noted in the initial test.

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[1] Spies, T. D., Gross, E. S., and Sasaki, Y., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 178.

[2] Mason, V. R., Courville, C., and Ziskind, E., *Medicine,* 1933, **12**, 355.

* The method of Beckh, Ellinger and Spies[3] was used for the determination of porphyrin in the urine. This method may be described briefly as follows: 10 cc. of urine were acidified with glacial acetic acid to pH 4 (about 6 drops) and then extracted in a separatory funnel with 10-15 cc. of ether. The ether extract was washed twice with 10-15 cc. of distilled water. After the second washing, the entire aqueous portion was discarded. To the ether extract was added ⅕ its volume of 25% hydrochloric acid. With thorough mixing, the ether soluble porphyrin enters the acid layer where it produces colors ranging from pink to purple, according to the concentration of porphyrin present.

[3] Beckh, W., Ellinger, P., and Spies, T. D., *Quart. J. Med.,* 1937, **6**, 305.

TABLE I.

Summary of Data and Treatment.

400 mg. nicotinic acid given on days indicated *. + indicates positive and — indicates negative test for increased porphyrinuria.

Days	1	2	*3	*4	5	6	7	*8	*9	*10	*11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
J.D.	+	+	+	+	+			+	+	—	—	—			—	+	+		+			+	+	+	+	+
A.G.	+	+	+	+	+			+	—	—	—	—			—	+	+		+			+	+	+		
W.J.	+	+	+	+	+			+	+	—	—	—			+	+	+		+			+	+	+	+	+
J.H.	+	+	+					+	+	—	—	—			+	+	—					+	+	+		
J.K.	+	+	+	+	+			+	—	—		—			—	—										+
E.L.	+	+	+	+	+			+	—	—	—	—			—		—					+	+	+	+	+
A.E.		+	+	+	+			+	+	—	—	—			—	—	—		+				+	+	+	+

After the amount of porphyrin occurring in the urine of these control subjects had been determined, the following studies were made on the 7 painters. Throughout the entire period of study the urine of each painter was tested daily for porphyrin. The first 3 days served as a control period. During the next 9 days, nicotinic acid (2 doses of 200 mg. each, dissolved in 200 cc. of water, and administered at 11:30 A. M. and 4:00 P. M.) was given on the days indicated in Table I. Each painter received a total amount of 2.4 gm. of nicotinic acid. The daily tests for porphyrinuria were continued for 14 days after the administration of nicotinic acid had been discontinued, in order to determine whether the porphyrinuria would return to the amount observed in the initial control period.

Except for a transitory flushing and feeling of warmth, which were obtained in all cases but in varying degrees in different persons, no subjective effects were noted with the oral ingestion of nicotinic acid in the amount administered in this study. The effect of the first dose was, however, more intense than were subsequent doses. This abnormal dilatation of the peripheral vessels reached a maximum about one-half hour after the oral administration of the nicotinic acid and disappeared in the following 2 hours. This effect has been noted by several investigators[4, 5, 6] and is not interpreted by the authors as being harmful.

During the 3-day control period, all of the painters consistently showed a strongly positive test for porphyrin in the urine, although there were variations among the individuals of the group. The day after the administration of nicotinic acid was begun, reductions in the amount of color produced were noted in all cases. After the 9-day period of nicotinic acid medication, the urines of all 7 painters gave no color or only traces of color. The administration of nicotinic acid was then discontinued. Within the next 14 days the amount of color gradually increased to the intensity observed during the initial control period.

In order to be certain that the presence of nicotinic acid in the urine was not giving false negative tests for increased porphyrinuria, varying quantities of nicotinic acid were added to samples of urine containing an increased amount of porphyrin. In no case was the intensity of the color altered by the addition of nicotinic acid. It was also found that urine obtained from an individual who had

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[4] Spies, T. D., Cooper, C., and Blankenhorn, M. A., read before the Central Society for Clinical Research in Chicago, Illinois, November 5, 1937.

[5] Smith, D. T., Ruffin, J. M., and Smith, S. G., *J. A. M. A.*, 1937, **109**, 2054.

[6] Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 405.

ingested large quantities of nicotinic acid did not obscure the test for porphyrins when it was added to urine with an increased quantity of porphyrin.

Twelve control subjects showed a transitory increased porphyrinuria, which, however, was not as great as that found in 7 painters before the administration of nicotinic acid.

The results summarized in Table I show that even in cases of long standing increased porphyrinuria such as that resulting from exposure to lead for periods of at least 15 years, nicotinic acid in the amounts given readily reduced the degree of porphyrinuria. This finding supports the observation of Spies, Gross and Sasaki[1] that the porphyrinuria present in pellagra and certain other diseases decreased rapidly following the administration of nicotinic acid. Since the porphyrinuria was reduced to its minimal quantities only after a number of days, rather than immediately after the nicotinic acid was administered, it seems that this substance does not mask the porphyrinuria but plays a rôle in the metabolism of porphyrin.

Summary and Conclusions. 1. The urine of 7 painters showed increased porphyrinuria as compared with the amount present in the urine of 45 control subjects. 2. In every instance this increased porphyrinuria decreased following the administration of nicotinic acid. 3. These studies suggest that nicotinic acid may play a rôle in the metabolism of porphyrins.

9824 P

A Complement-binding Antigen in Extracts of the Brown-Pearce Carcinoma of Rabbits.

John G. Kidd.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

Previous studies[1] have shown that a complement-binding antigen is present in extracts of virus-induced rabbit papillomas, and that this antigen is closely associated with the virus causing the growths, if not identical with it.[2] In the present work it has been learned that extracts of a transplantable epithelioma of rabbits[3] also contain a complement-binding antigen which will react specifically with the sera of rabbits bearing the tumor, or in which it has retrogressed.

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[1] Kidd, John G., Proc. Soc. Exp. Biol. and Med., 1937, **35**, 612.

[2] Kidd, John G., Proc. Soc. Exp. Biol. and Med., 1938, **37**, 657.

[3] Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, **37**, 601.

Procedures. The antigen-extracts were prepared from tumor tissue obtained from vigorous transplants in muscle or testicles, or from metastases in the omentum or diaphragm. The tissue was kept frozen at —20°C. or in 50% glycerol-Locke's solution at about 4°C. until needed, then ground with sand and suspended in isotonic saline (proportions usually 1:20), kept overnight in the refrigerator, and centrifugalized at 3500 r.p.m. in the angle-head centrifuge for 10 to 20 minutes. The supernatant fluids were then inactivated at 56°C. for 30 minutes and used in the tests.

Tests have been made using extracts of the Brown-Pearce tumors of 8 rabbits and sera from 34 animals bearing the growths or in which these had recently retrogressed. They were set up precisely as in the experiments with the papilloma virus, using 2 units of complement and sera diluted 1:4. Control tests for anticomplementary effect were made with double amounts of all materials.

Outcome of the Tests: Extracts of the vigorously-growing Brown-Pearce tumors of 7 rabbits bound complement notably well when mixed with sera of rabbits carrying the growths, but extracts of the necrotic tumor tissue of an 8th animal failed to do so.

Twenty of the 34 sera obtained from rabbits bled 28 days or longer after the tumor had been transplanted to them bound complement specifically when mixed with extracts of the growths, the most effective sera coming from rabbits which had borne 6 or 8 large tumors for some weeks.

None of 29 sera obtained from normal or pregnant rabbits, or from rabbits immune to vaccinia or Virus III, has bound complement specifically when mixed in the routine way with extracts containing the complement-binding antigen; nor has specific fixation occurred when active Brown-Pearce antisera were tested with extracts of rabbit embryos or of normal rabbit skin or testicles, or with extracts of rabbit testicles heavily infected with Virus III. In an experiment to test further the specificity of the reaction, the sera of 4 rabbits carrying the Brown-Pearce tumor bound complement when mixed with antigens derived from growths of this sort, but failed to do so when mixed with antigens derived from virus-induced rabbit papillomas; whereas 4 other sera, procured from rabbits immune to the papilloma virus, fixed complement in high titer when mixed with papilloma antigens, but did not do so at all when mixed with antigens derived from the Brown-Pearce tumors.

Properties of the Complement-Binding Antigen: Extracts of the Brown-Pearce tumor heated to 60°C. for 30 minutes retained their capacity to bind complement when mixed with active sera, but this was almost or entirely lost after heating them at temperatures of 66°C. or higher for the same period.

In many experiments the antigen has been found to pass through Berkefeld V filters, although some of it was retained by them as a rule. Most of it was held back by Berkefeld W candles, and practically all by single Seitz EK disks.

The complement-binding antigen has been deposited by centrifugation in two experiments. In the first, extracts of the Brown-Pearce tumors were put into a high-speed, angle-head International centrifuge, which was then run at about 18,000 r.p.m. for 6 hours; and in the second, similar extracts were spun in an air-driven ultracentrifuge at about 30,000 r.p.m. for 3 hours. The supernatant fluids in both cases were devoid of ability to bind complement when mixed with active antisera; whereas the sediments, resuspended in the original volumes of saline, had approximately the same capacity to fix complement as portions of the extracts which had not been centrifugalized.

To test the reaction of the complement-binding antigen to changes in pH, extracts of the omental metastases of 2 animals were prepared as usual, and portions of each were adjusted to the pH levels stated below with NaOH and HCl, some being saved untreated as well. All were incubated for 1 hour at 37°C., kept in the refrigerator overnight, inactivated at 56°C. for 30 minutes, and finally brought back to pH 6.8 to 7.0. When mixed immediately thereafter with active sera and tested for capacity to bind complement, those portions which had been subjected to pH 2.5 and 4.5 did not bind it at all; whereas the control portions, which had remained at pH 6.0 and 6.8 respectively, bound it completely. The materials subjected to pH 8.5 still bound complement, but not quite so well as did the controls; while those portions which had stood at pH 10.0 had little capacity to bind complement, and those kept at pH 11.5 had none whatever.

Tumor extracts containing the complement-binding antigen in large amount have failed to give rise to any lesions when inoculated intradermally or rubbed into the scarified skin of normal rabbits.

Summary and Comment: A substance is present in extracts of the Brown-Pearce tumor which binds complement specifically when mixed with the sera of rabbits bearing the growth, or in which it has retrogressed. The substance is readily distinguishable from the complement-binding antigen which can be extracted from the virus-induced papillomas of rabbits, though it resembles the latter in its general traits. Further work is under way to determine the significance of the findings.

9825 P

Germicidal Efficiency of Synthetic Phenolic Compounds Tested by the Improved Tissue Culture Method.

I. L. SHECHMEISTER AND A. J. SALLE.

From the Department of Bacteriology, University of California, Berkeley.

In the preceding paper of this series[1] an improved method was proposed for testing and rating germicides. The compounds which were rated by the method included: iodine, Hexylresorcinol, Metaphen, phenol, Mercurochrome, and Merthiolate.

The same method was followed for the evaluation of several synthetic organic compounds belonging to the phenolic group. The compounds were tested for their effect on the growth of living embryonic chick tissue as well as for their ability to kill bacteria. A number known as the toxicity-index was determined which is defined as the ratio of the highest dilution of disinfectant showing no growth of embryonic tissue in 10 minutes to the highest dilution required to kill the test-organism in the same period of time. The tests were run at a temperature of 37 C. in the presence of a standard amount of organic matter. Theoretically the smaller the index the more nearly perfect the germicide.

The following compounds were tested and compared: *o*-n-hexylphenol, *p*-hydroxydiphenyl sulfide, and *p*-hydroxyphenyl-n-amyl sulfide. Since phenol and Hexylresorcinol are also phenolic compounds they are included for comparison.

Effect of germicides on bacteria and tissue. The compounds were tested against both *Staphylococcus aureus* (gram +) and *Eberthella typhosa* (gram —). The killing concentrations of the germicides for tissue and bacteria and their toxicity-indices after 10 minutes' exposure at 37°C. are given in Table I.

The phenolic compounds that have been tested show relatively small toxicity-indices indicating that they are efficient germicidal

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[1] Salle, A. J., McOmie, W. A., Shechmeister, I. L., and Foord, D. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 694.

TABLE I.

Compound	Highest dilution showing no growth after 10 minutes' exposure			Toxicity-index = A/B	
	Staph. aureus = B	*E. typhosa* = B	Tissue = A	*Staph. aureus*	*E. typhosa*
Hexylresorcinol	1:1620	1:1770	1:1450	0.9	0.8
p-hydroxyphenyl-n-amyl sulfide	1:2700	1:2700	1:2800	1.0	1.0
o-n-hexylphenol	1:2900	1:2800	1:3125	1.1	1.1
p-hydroxydiphenyl sulfide	1:2500	1:3125	1:3125	1.3	1.0
Phenol	1:110	1:186	1:224	2.0	1.2

agents. Also, they exhibit about the same degree of germicidal efficiency against both *Staph. aureus* (gram +) and *E. typhosa* (gram —) making them valuable germicides for general use. With the exception of iodine the non-phenolic compounds which have been tested[1] show great differences in their action on *Staph. aureus* and *E. typhosa.*

9826

Relationship of Pyruvic Acid to the Bisulphite Binding Substances of the Blood.

ROBERT W. WILKINS, SOMA WEISS AND F. H. L. TAYLOR.

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston

Increases in the bisulphite binding substances (B.B.S.) of the blood have been related to increases in pyruvic acid, both in beriberi[1] and following exhaustive exercise.[2] The B.B.S. has been shown to be elevated in beriberi[1] and other disease states,[3] and also following the injection of sodium pyruvate.[4] The present communication gives a brief résumé of the relationship of the B.B.S. to the blood pyruvic acid.

Pyruvic acid was estimated in blood by Peters and Thompson's modification of the Neuberg-Case method,[5] B.B.S. by a modification[3] of the Clift and Cook procedure.[6] The methods used for selection of clinical material[3] and for administering sodium pyruvate[4] are given elsewhere.

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[1] Platt, B. S., and Lu, G. D., *Proc. Chinese Physiol. Soc.*, Third General Conference, Chinese M. A., 1935, p. 16; *Ibid., Quart. J. Med.*, 1936, n.s., **5**, 355.

[2] Johnson, R. E., and Edwards, H. T., *J. Biol. Chem.*, 1937, **118**, 427.

[3] Taylor, F. H. L., Weiss, Soma, and Wilkins, R. W., *J. Clin. Investigation*, 1937, **16**, 833.

[4] Wilkins, R. W., Weiss, Soma, and Taylor, F. H. L., *Ann. Int. Med.*, in press.

From pure aqueous solutions of lithium pyruvate an average of 85.7% of the theoretical amount of pyruvic acid as B.B.S. and 85.6% as pyruvic acid by the hydrazone method were recovered. The recovery of lithium pyruvate from blood, when added in amounts ranging between 10 and 20 mg. per 100 ml. of blood, averaged 90.4% of theoretical by the B.B.S. method and 79.9% by the hydrazone method. These values were obtained when the blood proteins were precipitated immediately after the addition of the pyruvate.

Thirteen subjects having a B.B.S. in the range previously accepted as normal[3] had blood pyruvic acid values between 0.19 and 0.79 mg. per 100 ml. Three patients with nutritional deficiency and with B.B.S. values of 6.6, 9.6, and 13.8, calculated as pyruvic acid in milligrams of pyruvic acid per 100 ml. of blood, had pyruvic acid values by the hydrazone method of 0.73, 0.46, and 0.72 mg. per 100 ml., respectively. In both normal subjects and those with elevated B.B.S. there was a much greater increase in B.B.S. than could be accounted for on the basis of the amount of pyruvic acid present.

B.B.S. and pyruvic acid were determined on the blood of 10 subjects before, and from one to 5 minutes after the slow intravenous injection of 5 gm. (100 cc. of a 5% solution) of pyruvic acid as sodium pyruvate. In each instance there was an increase in the circulating pyruvic acid and substances which bind bisulphite. From an average initial value of 0.37 mg., the pyruvic acid by direct hydrazone method increased to 4.48 mg., while the pyruvic acid by B.B.S. increased from an average value of 4.88 to 14.13 mg. per 100 ml. of blood. The average increase in pyruvic acid accounted for only 44.5% of the increase in B.B.S. expressed as pyruvic acid.

The foregoing observations were repeated on 5 individuals following the oral administration of 25 gm. of sodium pyruvate, with similar results when measurements were made before and from one to 2 hours following the administration of the sodium pyruvate. The essential difference was that the increase in pyruvic acid and B.B.S. in the blood was less than when given by vein. In these observations the increase in pyruvic acid in the blood accounted for

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[5] Peters, R. A., and Thompson, R. H. S., *Biochem. J.*, 1934, **28**, 916.

[6] Clift, F. P., and Cook, R. P., *Biochem. J.*, 1932, **26**, 1788.

54% of the increase in B.B.S. Following both oral and parenteral administration of sodium pyruvate positive tests for pyruvic acid were obtained in the urine for from 7 to 10 hours, and, in some instances, longer.

When blood to which lithium pyruvate had been added was permitted to stand at laboratory conditions for several hours, marked diminution in the recovery of pyruvic acid occurred. Under such circumstances the recovery of pyruvic acid by the B.B.S. method was only slightly reduced, while that estimated by the direct method was reduced to a much greater extent, 60% or less of added pyruvic acid being recovered by the direct method as compared with 80% when precipitation was carried out immediately.

A consideration of the above data suggests that pyruvic acid was converted to other substances of a ketonic nature by blood, both *in vitro* and *in vivo*. Additional *in vitro* experiments add some weight to this concept. Following the addition of 10 mg. of lithium pyruvate to 2 samples of the same blood, immediate precipitation was carried out in one, and the other incubated at 37.5°C. for 2 hours before precipitation. Recovery of 97.8% of the added pyruvate by B.B.S. and 86% by the hydrazone method was obtained from the non-incubated sample, while the incubated sample yielded 28.6% as B.B.S. and 18% as pyruvic acid after 2 hours. The experiment was repeated with the same results. When 0.5 ml. of 5% potassium cyanide solution was added to the blood, however, the B.B.S. fell from an initial recovery of 93.1% to 69.9%, while recovery by the hydrazone method fell from 90% to 10.3% in the 2 hours of incubation.

These experiments indicate that pyruvic acid is destroyed rapidly in blood, both *in vivo* and after withdrawal from the vein. Evidence is given that pyruvic acid as such may be converted to other ketone forms which, in turn, are more slowly metabolized. Inhibition by cyanide appears to effect the oxidation of these secondary ketonic bodies rather than the conversion of pyruvic acid into them. No attempt has been made in these studies to identify ketone substances in addition to pyruvic acid. The data further indicate that determinations of pyruvic acid on blood by the hydrazone method must be carried out immediately after the sample is drawn.

9827 P

Renal and Vascular Responses to Epinephrine Injections in Glomerular and Aglomerular Fish.

LOUIS A. TOTH. (Introduced by Henry Laurens.)

From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans.

In a previous publication[1] it was reported that epinephrine infusions in the dog resulted in either an oliguria or a polyuria, depending on the rate of infusion. From the work of Richards and Plant[2] and Winton[3] the hypothesis was advanced that the dilute epinephrine in the blood stream resulting from a slow infusion constricted the efferent arterioles, causing a polyuria; but with a more rapid infusion and a resulting higher concentration the afferent, or both the afferent and efferent, arterioles were constricted, causing an oliguria. The present experiments were designed to determine the rôle of the glomerulus in the urinary response to epinephrine.

Seventeen experiments were carried out on 15 toadfish *(Opsanus tau)*, an animal possessing aglomerular kidneys.[4] Twenty-eight experiments were performed on 24 puffers *(Spheriodes maculatus)* which possess glomerular kidneys. The animal was anesthetized with urethane in sea water and after anesthesia had occurred it was transferred to a more dilute urethane solution. A slow stream of oxygen bubbled through the mouth and out the gill slits to prevent asphyxia. One ureter was cannulated and the urine flow was determined by frequent observations of the movement of the meniscus in the cannula. The arterial blood pressure was determined in a number of experiments by means of a Hg manometer, 10% sodium citrate being used as the anticoagulant. The gonadal vein was cannulated for the injection of the solution. After determining a normal urine rate dilute sea water equivalent in volume to that of the epinephrine solution to be injected was given by vein for the control injection. The volume never exceeded 1 cc.

In the toadfish, doses of epinephrine varying from 67 to 6 micrograms per 100 gm. body weight had no specific effect on the urinary rate. The blood pressure increased, in one experiment the increase was from 12 to 18 mm. Hg and lasted for 40 minutes. The heart rate usually showed a slight increase after both the epinephrine and the control solutions, the increase not exceeding 10 beats per minute. In contrast, the glomerular kidney responded with a tremendous polyuria (as great as 500× the control rate) to doses of epinephrine that ranged from 242 to 3 micrograms per 100 gm. body weight. The larger increases in urinary rate after epinephrine occurred in those experiments in which the control rate was low. A significant and prolonged rise in the blood pressure occurred after the epinephrine, the rise in one experiment being from 6 to 14 mm. Hg and lasting for approximately 80 minutes. The heart rate fell markedly after the injection of the drug (from 120 to 60 beats per minute in the above experiment) but gradually rose to the control level. This fall in heart rate was interpreted by Lutz[5] as the response of an unbalanced parasympathetic mechanism in an organ lacking a sympathetic accelerator innervation. Control injections did not change the heart rate or blood pressure significantly.

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[1] Toth, L. A., *Am. J. Physiol.*, 1937, **119**, 140.

[2] Richards, A. N., and Plant, O. H., *Am. J. Physiol.*, 1922, **59**, 191.

[3] Winton, F. R., *J. Physiol.*, 1931, **73**, 151.

[4] Marshall, E. K., *Bull. Johns Hopk. Hosp.*, 1929, **45**, 95.

9828 P

On the Nature of Insulin Convulsions.

M. CAROLINE HRUBETZ. (Introduced by E. L. Scott.)

From the Department of Physiology, College of Physicians and Surgeons, Columbia University.

With the injection of doses of insulin just adequate to induce convulsions, the animal will usually exhibit only clonic convulsions, but if twice that dosage be given to this same animal, tetanic convulsions usually result. If the low sugar level be the sole cause of the convulsions, then why would their character change with the increased dose? With doses of insulin adequate to render an animal convulsive, or even sub-convulsive, animals, as well as man, exhibit evidence of a functionally modified nervous system long before the true blood sugar level has fallen to zero and before any convulsions appear. Some of the evidences of this functional modification are marked salivation, profuse sweating, pupillary changes (dilation just before the onset of the convulsions and hippus often accompanied by nystagmus during the seizure), marked restlessness and increased sensitivity to stimuli such as light or noise.

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[5] **Lutz, B. R., *Am. J. Physiol.*, 1930, 94, 135.**

The barbiturates are being employed both clinically as well as experimentally for the prevention of insulin convulsions. In a search for the mechanism for this action of the barbiturates, the question arose as to whether the drug modified the effect of a convulsive dose of insulin upon the blood sugar. Table I summarizes some of the results.

TABLE I.*
Blood Sugar Levels after Insulin (2 units per kilo) Plus Phenobarbital (40 mg./kg.) Injected Simultaneously. Normal-fed Rabbits.

Rabbit No.	Initial mg.	Time after injection in hours ½ mg.	1 mg.	2 mg.	3 mg.
444	79	27	27	14	27
452	112	40	40	16	28
451	97	59	16	10	16
1124	120	71	14	14	92

* Somogyi's Reagent No. 1 for the micro method was used. Peters and Van Slyke, *Quantitative Clinical Chemistry*, Vol. II, p. 466 (Somogyi, M.), Williams and Wilkins, Baltimore, 1935. All values below 27 mg. were obtained by extrapolation.

The resulting values approximate the true sugar level of zero as found by Dotti.[1] In no case, however, were there any signs either of convulsions, or even of the prodromal signs. Although no glucose was administered, none of these animals died despite the fact that the true blood sugar levels remained at or near the zero level for at least the 3-hour observation period. Apparently, the protective mechanism of the barbiturate is independent of the blood sugar concentration. This would seem to indicate either that the drug so affects the central nervous system that it does not respond to the low sugar concentration in the usual manner, or that insulin has some effect on the central nervous system which is unrelated or, at most, related only indirectly to the low sugar level.

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[1] Dotti, L. B., *J. Biol. Chem.*, 1934, **104**, 535.